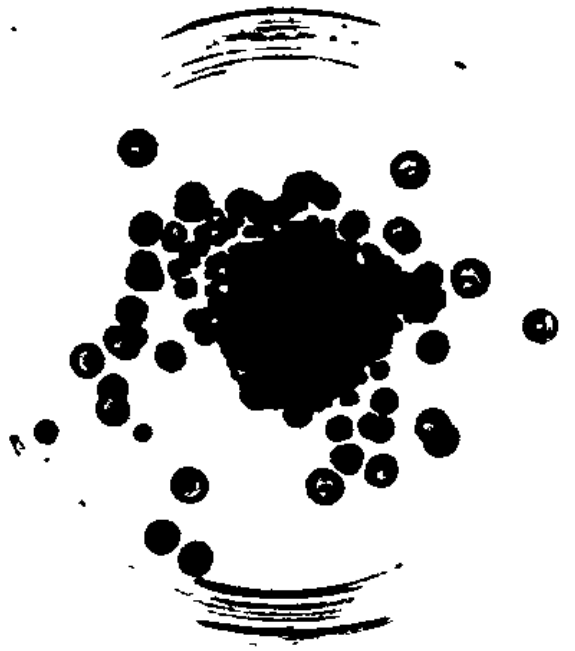

Streptomyces griseus

STREPTOMYCIN

Nature and Practical Applications

EDITED BY

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New Jersey Agricultural Experiment Station,
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PREFACE

Probably no other drug in the history of medical science has had such a phenomenal rise as streptomycin. The sulfa compounds, followed by penicillin, pointed to the great potentialities of chemotherapeutic agents, produced either by chemical synthesis or by certain microorganisms, in combating infections caused by bacteria and other microscopic and ultramicroscopic forms of life. These discoveries almost coincided with the reawakening of the general interest in antibiotics, or those substances which are produced by microorganisms and which have the capacity of inhibiting the growth and even of destroying other microorganisms, notably disease-producing bacteria.

Neither the synthetic sulfa drugs nor the antibiotics tyrothricin and penicillin, however, had sufficient activity upon some of the most important gram-negative bacteria or upon the acid-fast, notably the tuberculosis, organisms. Agents to combat these diseases were badly needed. The fact that the world was in the midst of a great catastrophe made the need for new chemotherapeutic agents more imperative. The isolation of streptothricin in 1942 demonstrated that such agents could be found and that the actinomycetes are probably the logical microorganisms which should be considered as potential producers of such agents. Streptomycin appeared to fill the gap.

In the five years since the isolation of streptomycin in 1943, considerable progress has been made, as evidenced by the fact that a literature of nearly 1,800 references, covering reports of investigations in many countries and languages, has accumulated This is ample justification for an attempt to summarize the present status of the subject. In the following chapters, outstanding authorities, many of whom have pioneered in the development and utilization of streptomycin, notably its isolation and clinical applications, have collaborated to present summaries of their work as well as that of others in the field. Each chapter is accompanied by only very few pertinent references For the more complete literature the reader is referred to "The Literature on Streptomycin, 1944–1948," published by the Rutgers University Press.

It is sincerely hoped that this information will prove useful to all those who are interested in streptomycin, especially in its use in combating bacterial infections

SELMAN A. WAKSMAN

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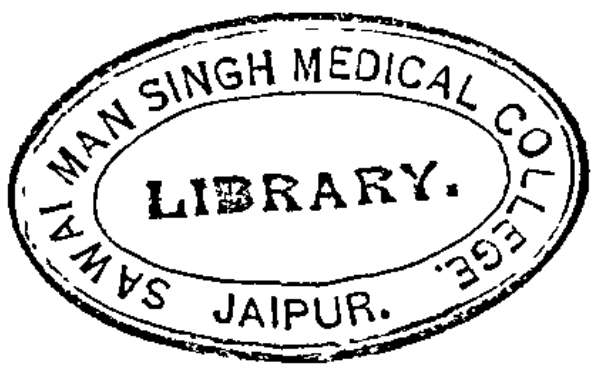
Section I

MICROBIOLOGICAL AND CHEMICAL ASPECTS



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CHAPTER 1

HISTORICAL INTRODUCTION

The organism producing streptomycin was first isolated in the laboratories of the Department of Microbiology of the New Jersey Agricultural Experiment Station, Rutgers University, in September 1943. The first public announcement of the isolation of the antibiotic was made by Schatz, Bugie, and Waksman (1) in January 1944. Its *in vivo* activity was soon established (2). Before the end of the year, its activity against the tuberculosis organism, both *in vitro* (3) and *in vivo* (4), had been demonstrated, and although available in only very small amounts, streptomycin was being submitted to clinical trials. In less than two years from the date of its isolation, extensive investigational work, comprising bacteriological, chemical, pharmacological, and clinical studies, had been accomplished, and the practical potentialities of streptomycin as a chemotherapeutic agent were definitely established

In presenting a comprehensive summary of the clinical uses of streptomycin before the conference on Antibiotics, held by the New York Academy of Sciences, in January 1946, Hinshaw and Feldman (5) of the Mayo Clinic, dedicated their address to the second anniversary of the announcement of the isolation of streptomycin. The following year saw the inauguration of a series of intensive clinical applications of streptomycin in the treatment of numerous diseases, mostly those caused by gram-negative bacteria or bacteria resistant to penicillin and to sulfa drugs. Hope was aroused, too, that possibly an agent had finally been found which was also effective against tuberculosis Several centers were established, during 1946, for testing the sensitivity of different freshly isolated strains of *M. tuberculosis* to streptomycin, and before that year came to an end, observations on the first hundred cases of tuberculosis treated with streptomycin were reported (6). The first observations were then made of the development of bacterial resistance to the drug and of the practical evaluation of this phenomenon in experimental animals. On February 17, 1947, in New York, a Conference was arranged by the American Trudeau Society and the National Tuberculosis Association to discuss the application of streptomycin to clinical tuberculosis, the methods of administration, and possible

purchase of streptomycin when its use was indicated. The requests of other institutions were given careful consideration, since each depot hospital was limited to a monthly quota. By the beginning of 1947, the Committee on Chemotherapy had completed its work. Enough streptomycin was then being produced to make possible wide domestic sales and even export. The various companies manufacturing streptomycin have thus contributed in many ways to the elucidation of this drug as a chemotherapeutic agent.

Finally, record must be made of the Conference on Tuberculosis held by the Interim Commission of the World Health Organization, on July 30–31, 1948, in New York, attended by representatives from various countries and at which definite recommendations were drafted concerning the use and value of streptomycin in tuberculosis.

Antagonistic Properties of Actinomycetes

The isolation of streptomycin and its utilization in the treatment of numerous infections in man and in animals which previously had not lent themselves to therapy, was a high point in a long and painstaking search for antibiotics, a search which is continuing unabated The microbes which produce streptomycin belong to the actinomycetes, a group of organisms occurring abundantly in soils, manures, composts, fresh water basins, and dust. They are filamentous and branching organisms that the bacteriologist has been accustomed to consider as bacteria and that the mycologist was inclined to classify with the fungi.

In attempts to elucidate the nature of the complex microbiological population of the soil and other natural substrates, the paramount fact that impresses itself upon investigators is that those microbes which occur in natural substrates exert a variety of associative and antagonistic influences upon one another Among the various groups of microorganisms, the actinomycetes appear to persist longest in the soil, especially under conditions unfavorable to the growth of other organisms, such as would result from drying, treatment with antiseptics, or after extensive and prolonged decomposition of organic materials.

The ability of a large number of actinomycetes to inhibit the growth of bacteria, fungi, and other actinomycetes had been established by many investigators studying this group of organisms. Gasperini first reported in 1890 (10) that certain actinomycetes, designated as *Streptothrix*, have the capacity to develop upon the surfaces of bacteria and fungi and to digest the membranes of these organisms At a much later date, in 1921, Lieske demonstrated that various actinomycetes were able to bring about lysis of many dead and living bacterial cells, and that the antibacterial activities of the actinomycetes were selective in nature, affecting only certain organisms, such as *S. aureus*, and not others. Soon afterwards, in 1924, Gratia

toxic effects. This event took place exactly three years after the public announcement of streptomycin reached the scientific reader.

The third year also brought about the almost complete elucidation of the chemistry of the streptomycin molecule, the preparation of the first chemical derivative of streptomycin, and the report of the first thousand clinical cases treated with this drug. Within four years streptomycin production had grown from a laboratory curiosity into a large industry, with a monthly output of more than 3,000 kg of the pure base.

This rapid progress in the development of streptomycin was due largely to two factors: the spectacular rise of penicillin between 1941 and 1943 which suggested the possibility of finding other antibiotics that could be utilized as chemotherapeutic agents for treatment of diseases not affected by penicillin, and the investigations carried out previously in the laboratories of the Department of Microbiology on streptothricin, which is similar chemically and biologically to streptomycin The work on streptothricin laid the foundation for the subsequent rapid progress in the isolation and use of streptomycin, the latter having a broader antibacterial spectrum and being less toxic to animals than the former.

The Committee on Chemotherapeutics of the National Research Council contributed much to the coordination of the clinical work done on streptomycin (8). At first, all the streptomycin produced was reported to the Civilian Production Administration for allocation. First consideration was given to the needs of the Army, Navy, U. S. Public Health Service, Veterans Administration, and the National Research Council, and the available supply was adjusted to those needs. No other agency was allowed to purchase streptomycin. No patient who was treated with this drug paid for it, and no physician was charged for it. The program of clinical research was thus conducted by the concerted efforts of the government, the producers of streptomycin, the National Research Council, and civilian medical scientists, with the sole purpose of obtaining in the shortest possible time the necessary information concerning this antibiotic The program of the National Research Council was supported by grants in aid from eleven pharmaceutical and chemical companies (9).

This program constituted the first privately financed, nationally coordinated clinical evaluation in history It was made possible by a joint contribution of nearly $1,000,000 from pharmaceutical and chemical manufacturers of streptomycin. It was estimated that, by October 1946, the manufacturing laboratories had invested $20,000,000 in production facilities for a drug of very recent origin.

Wide-scale distribution of streptomycin began in September 1946, when about 1,600 general hospitals were designated as depots for the drug. Any nondepot hospital or any physician could call upon a depot hospital for the

Production of Antibiotics by Actinomycetes

Previous to 1940, most of the investigations on the antibacterial activities of actinomycetes were limited to the living organisms. At that time, only one preparation which had antibacterial effects was known. This preparation, designated by Welsch as ACTINOMYCETIN, had the properties of a proteolytic enzyme, and was active primarily on dead bacterial cells.

The first experiments, begun in 1939, in the Department of Microbiology of the New Jersey Agricultural Experiment Station, on the production of antibacterial substances by microorganisms, led to the isolation of a culture of an actinomyces, which was later described as *A.* (*S.*) *antibioticus*. This culture produced a highly potent antibiotic, which was named ACTINOMYCIN. This antibiotic was soon crystallized, and its chemical and antibacterial properties were determined. Although interesting from a chemical and biological point of view, it did not offer any remarkable chemotherapeutic potentialities, since it was extremely toxic to the experimental animals.

Other substances were soon isolated, notably PROACTINOMYCIN and MICROMONOSPORIN. Neither of these appeared to offer any distinct possibilities as a chemotherapeutic agent. Since penicillin appeared on the horizon in 1941 and since it promised to fill the need for the treatment of diseases caused by gram-positive bacteria, it was decided to concentrate upon the isolation of antibiotics which would be active against the gram-negative bacteria and upon the acid-fast group of bacteria, including the tuberculosis organism. These studies in the New Jersey laboratories, and in others, resulted in isolation of a large number of antibiotic substances from actinomycetes. These substances are active not only against various bacteria, but also upon fungi and viruses. Special procedures had to be developed for the growth of each specific organism and for the production and isolation of each specific antibiotic More than thirty antibiotics have now been isolated or at least are known to be produced by actinomycetes.

A comparison of the antibacterial spectra of some of these antibiotics with those produced by fungi is given in table 1. Antibiotic spectra for STREPTOTHRICIN and STREPTOMYCIN are shown in table 2. The four antibiotics of the fungi possess a high activity against gram-positive bacteria and relatively little action on the gram-negative forms, the acid-fast organisms falling between. Streptothricin and streptomycin are highly active gainst the last two groups of organisms. These two antibiotics show certain similarities in their general antibiotic spectra, but they also exhibit marked differences, as shown in their specific effects upon *B. mycoides* and *B. cereus*, on the one hand, and upon certain gram-negative bacteria, as *S.*

also observed that cultures of *Streptothrix* were able to lyse bacterial cells; he used such lysed cells, designated as *mycolysates, for immunizing purposes.*

Other investigations soon followed. These brought out the fact that certain species, or perhaps strains, of actinomycetes have the capacity to inhibit the growth of various bacteria, as shown by Rosenthal for the diphtheria organism. Some of these actinomycetes were found capable of producing a thermostable active substance. This substance was also strongly bactericidal, but its activity was limited to certain bacteria; it had little effect upon others.

The wide distribution of antagonistic actinomycetes in nature was first established by Nakhimovskaia in 1937. Out of eighty cultures isolated from a variety of soils, forty-seven possessed antibacterial properties, but only twenty-seven liberated active substances into the medium. The activity of these substances consisted largely in inhibiting the growth of gram-positive bacteria; they had no effect upon gram-negative bacteria or fungi.

In a series of detailed surveys of the distribution of antagonistic properties among actinomycetes, Waksman *et al* reported, in 1942, the isolation of 244 cultures from various soils. Of these, 106, or 43.4 per cent, possessed some antagonistic properties, and forty-nine, or 20 per cent, were highly antagonistic. The nature of the test organism, the composition of medium, and the method of testing greatly influenced the results obtained. In another survey of 187 cultures, freshly isolated from different substrates, only 3 per cent gave a high activity against *E. coli* when tested on nutrient agar and 6 per cent on dextrose-asparagine agar; the corresponding figures for the cultures active against *B subtilis* were 16 and 44 per cent. This pointed to the limited activity of the antagonistic actinomycetes upon gram-negative bacteria, as compared to the gram-positive forms.

The ability of actinomycetes to antagonize fungi has also been established. A survey of the antagonistic properties of eighty cultures made by Alexopoulos, using the fungus *Colletotrichum* as the test organism, gave 17 5 per cent as strong inhibitors, 38 8 per cent as weak inhibitors, the others having no effect. The inhibiting action of certain soil actinomycetes upon plant pathogenic forms was also established, it has even been proposed that this phenomenon be utilized for the control of potato scab, a disease of potatoes caused by *S scabies.*

Thus by 1942, it was definitely established that a large proportion of the actinomycetes possessed remarkable properties of inhibiting the growth of various bacteria and of other microorganisms, and even of causing their destruction.

only against *M. smegmatis*, *M. phlei*, and the nonpathogenic strain 607 of *M. tuberculosis* and NOCARDIN, which is active against *M. tuberculosis*.

Other antibiotics, such as streptothricin, streptomycin, CHLOROMYCETIN, and AUREOMYCIN, have very wide antibiotic spectra, those of the last two affecting rickettseae and some of the larger viruses.

Some antibiotics are now known to be produced by more than one species of actinomyces. This was found to hold true for actinomycin, which is formed by *S. antibioticus* and by a number of other species belonging to the genus *Streptomyces*, and for streptomycin, which is produced by *S. griseus* and by *S. bikiniensis*. Some antibiotics, such as members of the streptothricin group, vary greatly in their chemical make-up, in their selective activity upon different microorganisms, and in their toxicity to animals. Certain actinomycetes produce more than one antibiotic. This is true, for example, of *Streptomyces* F, which produces streptomycin and streptothricin, and of *S. griseus*, which produces, in addition to streptomycin and mannosidostreptomycin, actidione and streptocin.

Isolation of Streptothricin

With the isolation of streptothricin in 1942 by Waksman and Woodruff (11), an antibiotic was obtained which showed distinct promise in activity against gram-negative bacteria, accompanied by a limited toxicity to animals. This substance was produced by a culture of an actinomyces, known as *A.* (*S.*) *lavendulae*, which was obtained from the soil, in the laboratories of the Microbiology Department of the New Jersey Agricultural Experiment Station in 1941. The name streptothricin was derived from *Streptothrix* given to the actinomycetes by Ferdinand Cohn, in 1875. Other strains of this organism or of closely related organisms were later isolated and found capable of producing this antibiotic.

Streptothricin was found to possess highly desirable physical, chemical, and antibacterial properties and offered promise as a chemotherapeutic agent Streptothricin is water-soluble, fairly resistant to heat, and is active over a wide pH range, with an optimum at a slight alkalinity. It is active against numerous gram-negative and certain gram-positive bacteria, both *in vitro* and *in vivo*. It is active also against fungi, but not against viruses. It is highly resistant to the action of different organisms; it cannot be destroyed by fungi, by bacteria, or by enzymes.

Streptothricin is formed also by certain actinomycetes in admixture with other antibiotics, as for example, with streptomycin. Its production takes place under both stationary and submerged conditions of culture. Actinomycetes capable of producing streptothricin or related compounds are widely distributed in nature, this explains the large number of compounds already isolated which have properties similar to those of this antibiotic

marcescens, on the other. Streptothricin is more active against fungi than is streptomycin. It is also more toxic.

TABLE 1

Antibacterial spectra of several antibiotics (12)

Minimum inhibitory concentration of antibacterial substances in micrograms per milliliter

	GLIOTOXIN	PENICILLIC ACID	PENICILLIN G	PENICILLIN X	STREPTOMYCIN	STREPTOTHRICIN
B. mycoides	0.25	32	30.00	30.00	0.13	100.0
B. subtilis	0.25	8	0.03	0.06	0.25	0.8
S aureus	0.15	16	0.016	0.03	0.03	0.1
E. coli.	25.00	64	14 00	14.00	0.25	0.3
Kl. pneumoniae	6.00	64	110.00	240.00	0.13	0.1
Ps. aeruginosa	500.00	1,000	500 00	500.00	4.00	2 0
M. phlei.	4.00	64	14.00	20.00	0.25	7.0
M. smegmatis	4.00	32	450.00	470.00	1.00	14.0

TABLE 2

Comparative bacteriostatic spectra of streptomycin and streptothricin

On basis of crude, ash-free dry material

ORGANISM	GRAM STAIN	UNITS OF ACTIVITY PER MILLIGRAM ASH FREE DRY MATERIAL	
		Streptomycin	Streptothricin
B. subtilis	+	500	500
B. mycoides	+	1,000	<3
B. cereus	+	120	<3
B. megatherium	+	400	150
S. aureus	+	60	200
S. lutea	+	400	150
M. phlei	+	400	50
M. tuberculosis	+	120	—
Ph. pruni	−	400	400
E coli	−	100	100
S. marcescens	−	100	5
A. aerogenes	−	40	50
Pr. vulgaris	−	40	50
Ps. fluorescens	−	8	<3
Ps aeruginosa	−	4	<3
Cl. butylicum	−	12	<3

Actinomycetes were thus found to be active producers of antibiotics that vary greatly in their antimicrobial spectra. Some of these spectra are very narrow, as in the case of the so-called ANTISMEGMATIS factor, which is active

tuberculosis; its biological properties are similar, however, to the parent streptomycin, as shown in table 3.

TABLE 3

In vitro activities of pure streptomycins against various organisms
(Modified from paper 13)

TEST ORGANISM	MINIMAL INHIBITING CONCENTRATION*			
	Streptomycin	Dihydrostreptomycin	Mannosidostreptomycin	Dihydromannosidostreptomycin
	μg/ml	μg/ml	μg/ml	μg/ml
Kl. pneumoniae (ATCC 9997)	1.76	1.76	6.39	6.59
A. aerogenes (ATCC 129)...	2 71	3.27	10.80	11.10
E coli (D 56)	6.05	6.79	24 80	23 80
S. schottmülleri (D 51)	10.10	36.50	14.30	14 40
S. typhosa (D 15)	12.20	51.00	12.40	12.90
S. enteriditis (D 61)	4 14	5.50	12 70	13.60
Sh sonnei (H 1414)	7 42	8.52	30 60	30.30
Sh. dysenteriae (H 141)	6 26	5.82	27 20	27.10
Br. abortus (Huddleson 1119 avirulent)	0 816	0 738	2.93	2.53
H influenzae type b (D 68)	2.30	1 53	8 53	5.53
M pyogenes (*Staphylococcus*) var *aureus* (209P)	0 828	1.39	5 64	7.77
S pyogenes (C203)	11 70	15.90	82 90	87 90
M. tuberculosis·				
H37Rv	2.00	2 20	5 50	6 50
Ravenel	0 58	0.62	2 50	2.20
BCG	0 52	0 55	1 90	1.70
N†	0 54	0 56	2 50	2 10
T†	0.55	0.54	2 20	2 00
P†	0 62	0 85	2 30	2.20
OD†	0 63	0 75	2 30	2 60
K†	1 00	1 70	3 90	3 90

* All figures are given in terms of weight of the trihydrochlorides On the basis of assays with *Kl pneumoniae*, the streptomycin and dihydrostreptomycin would have an activity of 820 units per mg, the mannosidostreptomycin an activity of 236 units per mg and the dihydromannosidostreptomycin 228 units per mg.

† Strains of *M tuberculosis* freshly isolated from human cases

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It is sufficient to mention STREPTIN, LAVENDULIN, ACTINORUBIN, STREPTOLIN, and ANTIBIOTIC 136.

When detailed studies of the pharmacology of streptothricin were undertaken it was discovered that this antibiotic leaves a residual toxic effect in the animal body. Its use for parenteral administration was, therefore, excluded. Its possible practical application may be limited to oral or topical administration.

ISOLATION OF STREPTOMYCIN

The experience gained in the study of the formation and isolation of streptothricin from cultures of actinomycetes proved to be highly important in planning a search for other antibiotic agents that would possess similar or even more desirable biological and chemical properties, such as a broader antibiotic spectrum and less toxicity to the animal body. After further extensive studies of many actinomycetes representing a great variety of species and strains, two cultures were found to yield the desirable antibiotic. These were isolated from the soil and from the throat of a chicken. They both belonged to a species described as *A. griseus*, the first representative of which was isolated in this country in 1916 from the soil. The generic name of the organism was changed by Waksman and Henrici in 1943 from *Actinomyces* to *Streptomyces*. To honor this new generic name, the new antibiotic was designated as streptomycin.

As previously noted, the two cultures of the streptomycin-producing organism were first isolated in September 1943. Because of the similarity of the new antibiotic to streptothricin, both in isolation procedures and in its antibiotic spectrum, rapid progress was made in the development of suitable methods for the growth of the organism, *S. griseus*, for the isolation of streptomycin, and for the evaluation of its antimicrobial properties. In January 1944, four months after the two fresh isolates of *S. griseus* were obtained, the isolation of streptomycin was announced.

Thus streptomycin came into being. Recent plans of the National Tuberculosis Association for publication of its monograph *Streptomycin in the Treatment of Tuberculosis in Man* and the appearance of several volumes, in foreign languages, dealing with the same subject, emphasized the fact that finally a chemical compound has been discovered which has remarkable chemotherapeutic properties against the "white plague" of mankind, and that the eradication of this dreadful disease may be at hand.

As this is being written, several papers have appeared in the November issue of the American Review of Tuberculosis in which it is reported that dihydrostreptomycin, a derivative of streptomycin, is much less toxic than streptomycin when given in comparable doses and for similar periods, and may, therefore, be preferable in the treatment of some types of clinical

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CHAPTER 2

STREPTOMYCES GRISEUS. NATURE AND NUTRITION

ISOLATION OF S. GRISEUS

A culture of an organism believed to be actinomycetic and designated as *A. griseus* was first isolated from the soil and described by Krainsky, in Russia, in 1914. Another culture believed to be similar to this organism was isolated from a California soil by Waksman and Curtis (1) of the Department of Microbiology, New Jersey Agricultural Experiment Station, in 1916. As these two isolations and descriptions took place on the eve of or during World War I, Krainsky's culture was not available for comparison with the American culture. Since only the published description of Krainsky's organism was known, and in view of the great variability of actinomycetes as a whole and the difficulty of making comparisons based upon meager descriptions of cultural characteristics, it is questionable whether the two cultures were identical. The American workers must have recognized the probable lack of identity between the two cultures, since they emphasized that "the color of the aerial mycelium [of the American culture] is somewhat lighter than that described by Krainsky."

In connection with the search for organisms capable of producing antibiotic substances, especially those active against gram-negative bacteria, two cultures of an organism were isolated by Schatz and Waksman in September 1942 and found to be very similar to, if not identical with, the culture originally isolated by Waksman and Curtis. These two cultures, designated as *18-16* and *D-1*, were identical, both in their ability to produce the same type of antibiotic substance and in their morphological and cultural characteristics. They were isolated in different rooms, in different buildings on the campus, and within two days of each other, thus excluding the possibility of one's originating from the other as a contaminant. Although both were potent producers of the antibiotic, they differed in vigor of growth and in the quantitative production of the antibiotic under different conditions of culture. At first, D-1 was the more active, but it gradually began to lose its potency on continued cultivation in artificial

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produce streptomycin. Recently, Kelner (2) obtained by the irradiation of the 1915 culture of *S. griseus* a mutant which produced streptomycin. This mutant was in every other respect similar to the streptomycin-producing cultures isolated in 1943. This suggests the possibility that the original 1915 culture was also able to produce streptomycin but had lost this capacity on continued cultivation for 30 years upon artificial media.

When one of the streptomycin-producing cultures of *S. griseus*, No. 18-16, was plated out and individual colonies were picked and tested, they showed considerable variation in their ability to form streptomycin. Several of the strains derived from these colonies and designated by numbers are now in general use. Some of these strains, such as No. 4, were found to be more active in certain laboratories, and others, as No. 9, gave better yields of streptomycin in other laboratories. Differences were later observed also in the relative sensitivity of these two strains to the actinophage, No. 9 being the more susceptible.

By further strain selection, still greater increases in production of streptomycin were obtained. This selection was aided by irradiation with ultraviolet light or by x-ray mutations. Substrains which are yielding 400 to 500 μg/ml, and even as high as 1,000 μg/ml, of streptomycin were thus obtained from the original cultures, which gave an activity of only 100 to 200 μg/ml. The nature of the medium is of great importance, different strains giving higher yields on certain media than on others. This is also true of the medium used for the preliminary preparation of spore material or of mycelial growth prior to inoculation of the large fermenters.

The cultures of *S. griseus* are kept either in a lyophilized state or are first grown on soil and then dried down. They may also be kept on ordinary agar media, but must be transferred frequently.

Identification of Streptomycin-producing Cultures

Several procedures have now been developed for the isolation from natural substrates and for the identification of streptomycin-producing cultures, notably members of the genus *Streptomyces*, and specifically streptomycin-producing strains of *S. griseus*. These methods are based on certain properties of the organisms or on the nature and activities of the streptomycin formed by them.

1. Tolerance of fairly high concentrations of streptomycin in the medium. When a soil or other natural material is plated out on a medium containing 50 μg/ml of streptomycin, the great majority of bacteria and actinomycetes will fail to develop on the plate. With the exception of fungi, most of the actinomyces colonies will be of the *S. griseus* type.

2. Ability of certain resistant strains of test bacteria to grow in presence of streptomycin. Following the isolation of cultures from natural sub-

media. Culture 18-16 retained its high activity and yielded highly potent strains; it became the progenitor of all the cultures now being used for industrial production of streptomycin (fig. 1).

Many other cultures of *S. griseus* have since been isolated from soils, river muds, animal excreta, water, dust, and other natural substrates.

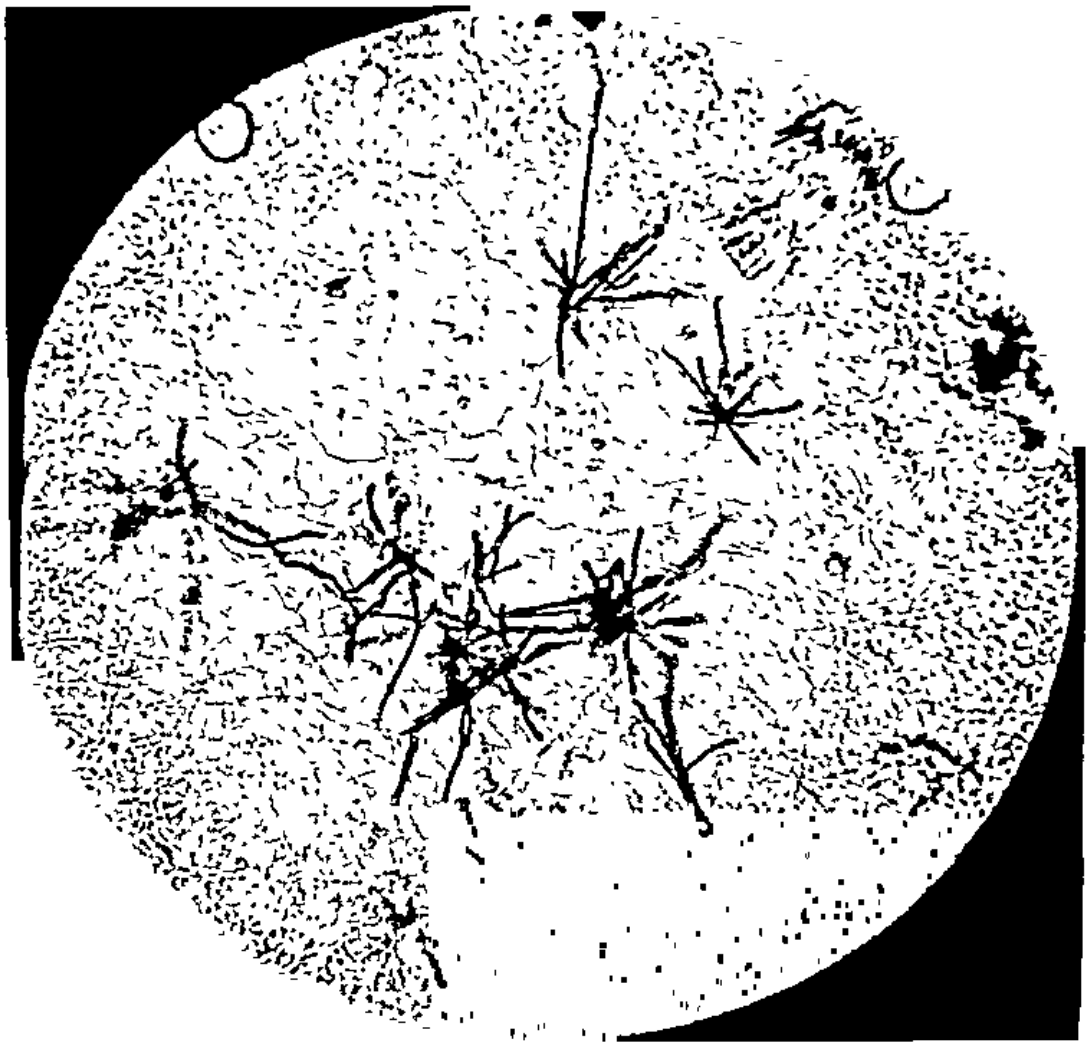

FIG. 1. *Streptomyces griseus*, streptomycin-producing strain. Vegetative and aerial mycelium (Original).

Not all of them were found capable, however, of producing streptomycin, the majority were either inactive or produced other antibiotics, such as grisein. Some of the cultures yielded a mixture of streptomycin with other antibiotics. The ability to produce streptomycin may, therefore, be considered as a strain, rather than a species, characteristic. In addition to *S. griseus*, certain other actinomycetes, such as *S. bikiniensis*, are also able to

strate, they are tested for their antibiotic-producing properties or antibiotic spectrum. Use of streptomycin-resistant test bacteria will indicate at once whether the culture produces streptomycin or whether it forms another antibiotic, such as streptothricin or chloromycetin. This is merely a special application of the antibiotic spectrum idea which has been used successfully for characterizing antibiotics.

3. Sensitivity to actinophage. When the *S. griseus* strains thus isolated are tested for their sensitivity to a specific actinophage, which is active only upon the streptomycin-producing strains, the inactive strains or those producing other antibiotics can be easily eliminated.

4. Utilization of streptomycin-dependent strains of bacteria in testing for streptomycin. When a culture of *S. griseus* or of another organism suspected of producing streptomycin is finally selected and grown in a liquid medium, the streptomycin-like nature of the antibiotic can be established by adding the culture filtrate to a nutrient broth and inoculating the latter with a streptomycin-dependent strain of *E. coli* or of some other bacterium. Growth of the bacterium will definitely establish the fact that the unknown antibiotic is streptomycin.

5 Cross-streaking the unknown cultures on a suitable agar medium toward known streptomycin-producing cultures. The latter will exert only a slight inhibiting effect upon the unknown streptomycin producers.

6. The Sakaguchi use of the test for establishing whether the basic compound, isolated in a crude state by suitable adsorption and elution procedures, is of a streptomycin or a streptothricin type.

Usually some soil or other material is plated on ordinary agar media favorable to the development of actinomycetes; colonies are picked and tested. The *S griseus* colonies can easily be recognized by the pale green to grayish green shade of their aerial mycelium (3) A suitable agar medium may also be seeded with living cells of a nonpathogenic strain of *M. tuberculosis*, and various dilutions of soil used for plating purposes. The plates are first incubated at 28° to 30°C for 2 or 3 days, to enable the actinomycetes to develop, this is followed by further incubation of the plates at 37°C for the development of the *M. tuberculosis*. Colonies that have the capacity of inhibiting growth of the bacterium are found to be surrounded by clear zones. By use of this method, Woodruff and Foster (4) isolated an organism which produced an antibiotic, designated as STREPTIN, but which was similar to streptothricin and not to streptomycin.

VARIATIONS AND MUTATIONS OF S. GRISEUS

The antibiotic potency of an active culture of *S. griseus* is fairly constant in spite of its ability to give rise to inactive variants. Highly active strains tend to retain their relatively superior streptomycin-producing potency

whereas poor strains usually remain weak producers of this antibiotic. For the commercial production of streptomycin, however, it is essential to select continuously the most active strains, as pointed out previously.

The formation of streptomycin takes place both under stationary and under submerged conditions of culture. Certain strains are more active under one set of conditions, and others under the other. Although use of

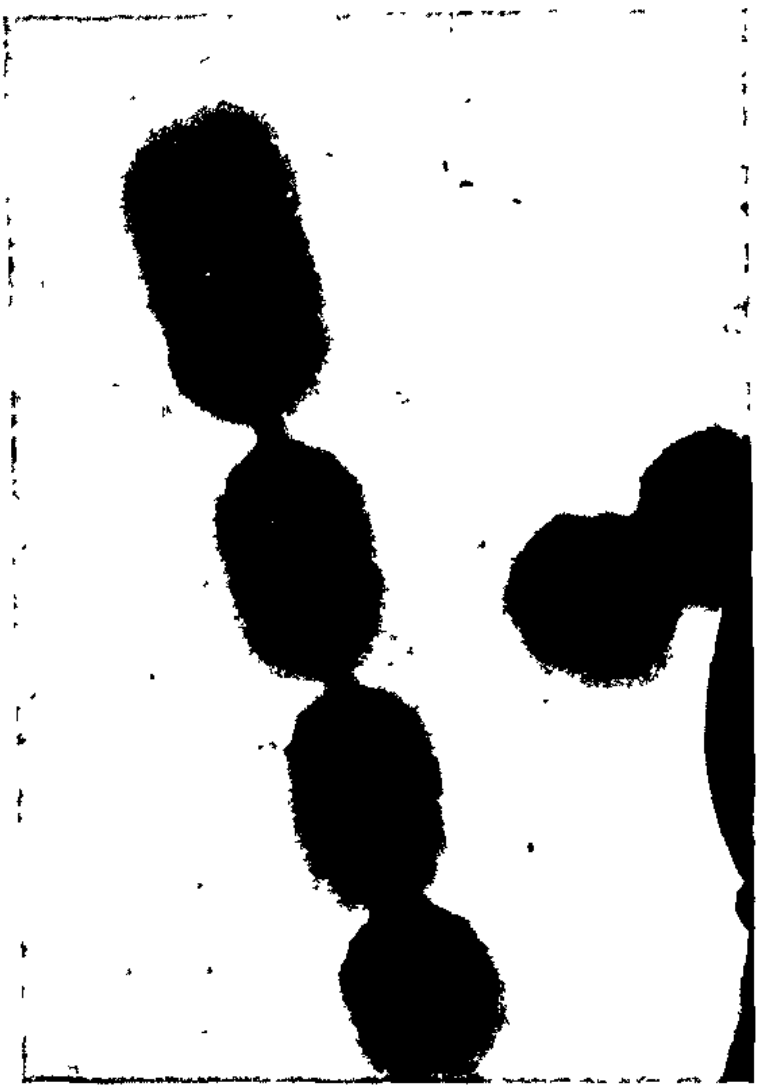

FIG 2. Electron microscope picture of *S. griseus*, showing spore formation (Courtesy of Schenley Dist Corp)

spore material for inoculation of the broth for streptomycin production presents less danger of degeneration of the culture than continuous use of the vegetative growth or the mycelium, repeated transfers of the latter are more convenient and even more satisfactory for large-scale fermentations. As many as ten mycelial transfers may be made in the process of inoculation of large fermenters.

S griseus undergoes in culture, under submerged conditions, a typical cycle of growth. The spores (fig. 2) first germinate and produce abundant

vegetative mycelium. The latter then undergoes a process of vacuolation, fragmentation, and cellular disintegration, accompanied by formation of large numbers of spores. These are produced under submerged conditions in shorter time and in larger numbers than on the aerial mycelium growing on the surface of solid or liquid media. The mycelial fragments are also capable of germinating and developing into a new mycelium. Spore production begins within 24 hours after submerged cultures are inoculated. The spores are formed in chains, either in sporogenous branches or at the tips of vegetative mycelium, in the main axis, and in secondary branches. The physiological and morphological properties of the spores produced under various conditions of culture are similar.

When the growth of *S. griseus* in submerged culture reaches a maximum, lysis sets in and fragmentation of the mycelium occurs. The peak of streptomycin production lags somewhat behind the growth peak. The changes in reaction of medium take place in two phases. One occurs during growth when the medium becomes acid, and one is associated with lysis of the culture, when the medium becomes alkaline and may reach a pH of 8.6.

S. griseus may give rise to two types of inactive strains. The first is free from aerial mycelium. In culture, especially in a submerged condition, it undergoes rapid lysis. It gives rise to an acid reaction in the medium, and yields a viscous broth. This strain is sensitive to the antibiotic action of streptomycin, and is comparable in that respect to other inactive actinomycetes, whereas the streptomycin-producing strains are highly resistant to the action of this antibiotic. This variant is similar to the active culture in cultural characteristics such as lack of dark pigmentation on organic media, proteolytic action, and hemolytic capacity. This asporogenous strain can be made, by proper culture and selection, to revert to the sporulating strain, which will have the capacity of producing streptomycin. The second variant is characterized by a pink pigmentation of the vegetative growth. It is unable to form streptomycin, although it produces another antibiotic, which is pigmented in nature, is soluble in organic solvents, and is highly active on gram-positive bacteria.

Activity of Actinophage upon S. griseus

The streptomycin-producing strains of *S. griseus* are subject to the action of a virus or phage which destroys both the mycelium and the spores of the organism (fig. 3). This phage has been designated as actinophage. When a culture becomes infected with the phage at an early stage of growth, it will undergo lysis with a corresponding loss in the production of streptomycin. By use of the plaque method, it is possible to measure the concentrations of actinophage in the culture. The number of phage particles per milliliter of preparation can reach 4×10^{10} to 1×10^{11}.

Phage-resistant streptomycin-producing strains of *S. griseus* can be

readily obtained, although they are not absolutely free from phage. The active culture is thus capable of continuously combating the attack by actinophage through the development of phage-resistant strains. However, the danger from culture destruction by phage in large-scale production of streptomycin is always present. *S. griseus* phage is not active upon other actinomycetes or upon strains of *S. griseus* that do not produce any streptomycin.

FIG. 3. Electron photograph of actinophage of streptomycin-producing *S griseus* (5)

Actinophage has its optimum temperature at 28°C. It does not multiply at 37°C or above. It withstands a temperature of 75°C, or somewhat above that which is injurious to the mycelium and spores of the organism (70°C). It is destroyed at 100°C in 10 minutes.

DESCRIPTION OF S. GRISEUS

S. griseus is characterized by certain morphological and cultural properties that make possible its identification and ready distinction from other

vegetative mycelium. The latter then undergoes a process of vacuolation, fragmentation, and cellular disintegration, accompanied by formation of large numbers of spores. These are produced under submerged conditions in shorter time and in larger numbers than on the aerial mycelium growing on the surface of solid or liquid media. The mycelial fragments are also capable of germinating and developing into a new mycelium. Spore production begins within 24 hours after submerged cultures are inoculated. The spores are formed in chains, either in sporogenous branches or at the tips of vegetative mycelium, in the main axis, and in secondary branches. The physiological and morphological properties of the spores produced under various conditions of culture are similar.

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was reported from California, Oregon, and Texas soils. Since streptomycin-producing strains are resistant to streptomycin, a medium containing this antibiotic leads to elimination from the plate of nearly all the bacteria and actinomycetes, and only *S. griseus* colonies develop profusely on such plates. This, accompanied by other procedures listed previously, makes possible not only isolation of streptomycin-producing strains of *S. griseus* but also their rapid identification and study of their distribution.

The question has often been raised whether there are several forms of streptomycin, as there are different penicillins. Two forms of streptomycin are now known, namely, the regular streptomycin and the mannosidostreptomycin. These differ in their quantitative antibacterial effect, but do not differ in their qualitative antibiotic spectra, nor do they seem to show any differences in their *in vivo* activity, aside from the quantitative effect

Growth and Metabolism of S. griseus

The course of growth of *S. griseus* in culture media can be measured by several different methods: (a) microscopic observations, (b) determination of weight of mycelium, (c) measurement of the nitrogen content of mycelium, (d) study of changes in the chemical composition of the medium, especially the carbohydrate and nitrogen constituents, (e) other measurements, such as increase in viscosity or change in pH of the medium.

Gottlieb and Anderson (8) studied the course of spore germination and of development of the mycelium in submerged culture. The exact time of spore germination was difficult to determine, only an elongation of the spores being observed. After 6 hours, the mycelium was found to consist of some small hyphae and of longer branched hyphae which tended to develop into masses of mycelium consisting of a dense solid center and a periphery of branched radiating hyphae. Within 24 to 30 hours, the entire body of the medium was filled with these mycelial clumps. The culture appeared viscous at this stage. After 48 hours, the mycelium began to fragment and spores were being produced. At 84 hours, definite lysis of the mycelium took place; the dense central core of the masses of growth disintegrated into granular pieces. Measurement of viscosity and weight of mycelium revealed an increase, which reached a maximum at 24 to 30 hours, followed by a decrease up to about 96 hours; a gradual leveling of growth then took place.

Although *S. griseus* can grow well on a variety of synthetic and organic media, production of streptomycin takes place only in certain organic media or in synthetic media of special composition. The first medium used for this purpose and designated as *No. 3* has the following composition:

Glucose	10 gm
Peptone	5 gm

members of the genus *Streptomyces*. As more and more cultures of *S. griseus* were isolated, it became recognized that this is a large group of organisms, the members of which vary greatly in their physiological properties, notably in the production of streptomycin and other antibiotics.

Waksman and Curtis described *S. griseus* as producing on Czapek-Dox agar a thin, spreading growth, developing deep into the medium, at first colorless, then turning olive-buff. This pigment may be lost on successive transfers. The aerial mycelium is thick, powdery, water-green in color. No soluble pigment was observed; the reverse of the growth became brownish in 24 days. On gelatin, at 18°C, *S. griseus* produces a greenish yellow or cream-colored growth developing deep into the substrate; the aerial mycelium is white-gray with a greenish tinge. There is no soluble pigment; liquefaction of the gelatin is rapid. *S. griseus* is capable of utilizing a variety of carbohydrates, including pentoses, hexoses, sugar alcohols, and organic acids. It is also able to obtain its nitrogen from a variety of compounds, including both inorganic and organic forms.

The first comprehensive study of the morphology of this organism was made by Drechsler (6). The aerial mycelium showed proliferations of fertile branches at moderately close intervals along the axial hyphae, but no spirals were produced. Occasionally, however, the production of a few close spirals was observed on certain media

In recent studies of the streptomycin-producing strains, the morphology and life-cycle of *S. griseus* were characterized in greater detail by Carvajal (7). The vegetative mycelium when young is well branched, typically in a monopodial form. Transverse septa are formed in virtually all cases in the delimitation of the reproductive cells Reproduction occurs by means of unicellular asexual spores and conidia, which are exogenously borne in chains on the aerial mycelium. The spores are of various shapes· barrel, oval, bean, spherical, and cylindrical Differences in shape and size are found often, even among the spores of the same chain Mature aerial spores often show small fragments of transparent film adhering to the outside wall The spores germinate at one end or at both ends, usually from the points at which they are attached to the adjacent spores or to the hypha Hyphal fusions and germ tube fusions also can be observed. A nucleus has been demonstrated in the germ tubes of *S. griseus* in the young mycelium, and in the developing spores. The nuclei are well distributed throughout the cytoplasm of the mycelium. The spores may be uninucleate or multinucleate

Distribution of S griseus in Nature

S. griseus represents a group of organisms widely distributed in the soil and in other natural substrates This was brought out in the earlier studies on the distribution of actinomycetes, when the isolation of this organism

was reported from California, Oregon, and Texas soils. Since streptomycin-producing strains are resistant to streptomycin, a medium containing this antibiotic leads to elimination from the plate of nearly all the bacteria and actinomycetes, and only *S. griseus* colonies develop profusely on such plates. This, accompanied by other procedures listed previously, makes possible not only isolation of streptomycin-producing strains of *S. griseus* but also their rapid identification and study of their distribution.

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Glucose	10 gm
Peptone	5 gm

Meat extract	5 gm
NaCl	5 gm
Tap water	1,000 ml

Other media containing casein digest, yeast extract, corn steep liquor or solids, and soybean meal were used later with highly satisfactory results.

Growth of *S. griseus* in stationary cultures reaches a maximum in 10 days, whereas maximum growth in submerged cultures is usually attained in 3 to 5 days. This is followed by lysis of the mycelium. Growth of the organism is accompanied by a gradual rise in pH value of the culture and in the ammonia and amino nitrogen contents; the total nitrogen in the mycelium tends to be higher during the active stages of growth. The production

TABLE 4

Metabolic changes characterizing the two phases during the submerged growth of S griseus (8)

	PHASE I	PHASE II
Streptomycin	Slight production	Maximum rate of production
pH	Gradual rise	Reaches maximum
Mycelium	Rapid growth	Gradual autolysis
Glucose	Rapid utilization	Small remaining amount exhausted
Soluble carbon	Gradual utilization	Concentration reaches maximum and remains constant
Lactic acid	Slow production and utilization	Slow utilization
Oxygen demand	Maximum	Decreases to minimum
Soluble nitrogen	Used extensively	Concentration increases
Inorganic phosphorus	Used at maximum rate	Released into medium

and accumulation of streptomycin parallels the growth of the organism, reaching a maximum when lysis just sets in, this is followed by a decrease when the rate of lysis reaches a maximum *S griseus* rapidly assimilates the phosphate in a phosphorus-poor medium. An excess of phosphorus has a depressive effect both upon growth of the organism and upon streptomycin production

The metabolic changes of *S. griseus* in a glucose-peptone-meat extract medium have been found by Dulaney and Perlman (9) to fall into two phases, as illustrated in table 4. During the first phase, the organism grows rapidly and forms extensive mycelium; this is accompanied by a reduction in the soluble constituents of the medium, namely the nitrogen, the inorganic phosphate, and the available carbohydrate; the quantity of lactic acid present is first increased and then utilized to some extent; the oxygen demand is high, and the QO_2 values may reach 150, little streptomycin is

produced; the soluble carbon content of the medium during the growth phase rapidly falls as the glucose is utilized; about 50 per cent of the carbon appears to be unavailable to the organism during the first stage; the nitrogen content of the mycelium varies with age. During the second or autolytic phase of growth considerable lysis sets in; streptomycin is produced actively, and the pH of the medium rises; the quantity of mycelium is decreased as a result of lysis; the lactic acid content remains more or less constant, as does the soluble carbon content of the medium; the oxygen demand slowly decreases; the ammonia nitrogen, soluble nitrogen, and inorganic phosphate contents of the medium rise rather markedly, paralleling the autolysis of the cells.

Ammonium compounds, but not nitrates, are favorable sources of nitrogen for growth and streptomycin production. A simple synthetic medium of the following composition has thus been devised:

Glucose	10.0 gm
$(NH_4)_2HPO_4$	4.0 gm
NaCl	5.0 gm
K_2HPO_4	2.0 gm
$MgSO_4 \cdot 7H_2O$	1.0 gm
$CaCl_2$	0.4 gm
$FeSO_4 \cdot 7H_2O$	20.0 mg
$MnSO_4 \cdot 7H_2O$	10.0 mg
Distilled water	1,000.0 ml

The supplementary addition of amino acids or of more complex organic compounds has been found to stimulate production of streptomycin. Among the amino acids, Eiser and McFarlane (10) found that histidine is essential both for mycelium and for streptomycin production; inositol also increased the yield of both; valine favored the latter, and aspartic or glutamic acid the former. If the salt concentration is low, most of the streptomycin will be found in the mycelium, thus suggesting that streptomycin is a product of intracellular synthesis. Woodruff and Ruger (11) reported that yields as high as 1 gm of streptomycin per liter are produced by *S. griseus* in media containing proline as the only source of nitrogen (fig. 4).

In recent studies of simple media, it has been found that the complex organic material is not essential and can be replaced by its ash, the potassium salt being the most significant constituent.

Actinomycetes are capable of producing lactic acid. *S. lavendulae*, for example, gives rise to fairly large amounts of this acid, provided sufficient carbohydrate is present in the medium; the pH changes to 5.7 in the presence of 5 per cent glucose (some actinomycetes produce enough acid to reduce the pH as low as 4.6). Lactic acid is not found in any large concentrations in the cultures of the sporulating strains of *S. griseus*, the aerial

mycelium-free mutants being capable, however, of forming considerable amounts of this acid.

S. griseus produces penicillinase and a variety of other enzymes. The ability of streptomycin-producing cultures of *S. griscus* to form an enzyme (mannosidostreptomycinase) which decomposes mannosidostreptomycin into streptomycin and mannose has been recently demonstrated (12). This enzyme is not produced by other actinomycetes or fungi.

The course of production and the chemical nature and antibacterial properties of streptomycin are treated elsewhere in this book.

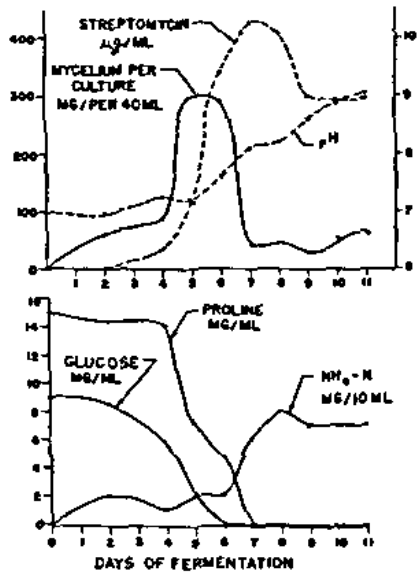

FIG 4. Effect of proline on growth and streptomycin production of *S. griseus* (11)

CHEMICAL COMPOSITION OF S.GRISEUS

On a dry basis, the mycelium of *S griseus* contains about 16 per cent ether-soluble material and about 37 per cent cold-water-soluble substances. Little study has been made of the specific chemical composition of these and other fractions Stokes and Gunness (13) grew *S. griseus* in stationary cultures in a nutrient medium containing 0 5 per cent meat extract and 1 per cent glucose. The cell material was dried and then hydrolyzed by acid or alkali The amino acid composition of this material, on a percentage basis of the dry material, was as follows: total nitrogen, 9.14; histidine, 0 84; arginine, 2.90, lysine, 2 13, leucine, 3 73, isoleucine, 1 49, valine, 3 40, methionine, 0 55, threonine, 2 33, phenylalanine, 1.67, tryptophane, 0.62.

The presence of glucose in the medium had a marked effect upon the amino acid composition of the mycelium in absence of glucose, the mycelium of *S. griseus* contained about twice as much histidine and arginine and much larger amounts of lysine, leucine, and threonine.

TABLE 5

Different antibiotics produced by various strains of Streptomyces griseus

ANTIBIOTIC	SOLUBILITY						C.I	Ph	ANTIBIOTIC SPECTRUM				FUNGI OR TRICHOMONADS
									Gram-positive		Gram-negative		
	Ac	Eth	E	A-Al	Cl.	W			*B. sub*	*B. myc.*	*E. coli*	*A. aer.*	
Streptomycin	i	i	i	s	i	s	+	+	+	+	+	+	−
Grisein	s	s	s	i	i	s	−	−	+	−	+	−	−
Actidione	s	s		s	s	s			−	−	−	−	yeasts
Streptocin	s	s	s	i	i	i			+	+	−	−	trichomonads
No. 3510 .	s	s	s	i	i	s	−	−	+	−	+	−	−
No. 3495 (pink)	s	s	s	i	s	i		−	+	+	−	−	−
No. 3522 (Baarn) .			sl	sl	sl			−	+	+	slight		−

Ac = acetone
Eth = ethanol
E = ether
A-al. = acid alcohol
Cl. = *chloroform*
W = water
C.I. = cysteine inactivation
Ph = phage = sensitivity to the actinophage active against streptomycin-producing strains.
sl = slightly soluble
s = soluble
i = insoluble

Formation of Other Antibiotics by S.griseus

In addition to the two forms of streptomycin, *S. griseus* produces several other antibiotics (table 5). Ether extracts from the mycelium of the organism a substance designated as STREPTOCIN, which is active against gram-positive bacteria, but not against gram-negative forms. Another antibiotic, designated as ACTIDIONE, was isolated (14) by extracting the crude submerged culture with chloroform; the extract was evaporated and the residue dissolved in methanol. Actidione is not active against bacteria but has strong antifungal properties; it is particularly active against yeasts and the pathogenic *C. neoformans;* it crystallizes from amyl acetate in the form of colorless plates, m.p. 115–116.5°; its composition agrees with the formula $C_{27}H_{42}N_2O_7$.

The culture filtrate of *S. griseus* was found (15) to possess certain antitoxic properties designated as ANTIDOTIC. The culture is able to destroy relatively large concentrations of various bacterial toxins, such as those of staphylococci and diphtheria, but not that of plague. Purified streptomycin does not possess such properties. The antidotic action of the filtrate of *S. griseus* is relatively stable to heat; its destruction begins at about 75°C.

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CHAPTER 3

STRAIN SELECTION AND STRAIN SPECIFICITY OF S. GRISEUS

With the possible exception of *A. fumigatus*, no microorganism has shown the prolific antibiotigenic character of *S. griseus*. In addition to the strains which have been shown to produce streptomycin (1, 2) and actidione (3), still others have been isolated which produce grisein (4); an antibiotic no. 3510, which is grisein-like (5); and two recently announced antibiotics (6) of which the most striking common identifying characteristic is their lack of activity against gram-negative bacteria. One of these is produced by Rutgers culture collection no. 3195, a pink mutant of a streptomycin-producing strain. The other is produced by a transfer from the original 1915 New Jersey culture which had been stored in Baarn, Holland, since 1921. This so-called "Baarn variant" now bears the R.C.C. no. 3522. Trussel, Fulton and Grant (7) have isolated a *Streptomyces* which produces two antibiotics, one of which appears to be streptomycin. The culture, still unidentified, does not key out to be *S. griseus*. Rickes, Brink, Koniuszy, Wood and Folkers (8) have found vitamin B_{12} in the culture medium of a grisein-producing strain of *S. griseus*.

This flood of data has caused Waksman to start grouping the various cultures with a view toward possibly defining new species within what seems to be a variable group of microorganisms. The absence, with few exceptions, of any morphological differences, together with the extreme variability of such physiological characteristics as have been tabulated, have so far made subdivision of this group unjustifiable. The very instability or extreme variability of *S. griseus* which causes so many taxonomic headaches makes it a promising microorganism for studies in strain selection and undoubtedly helps to explain why such an array of important metabolites have already been discovered.

Of all the antibiotics produced by *S. griseus*, streptomycin has become the most important. With few exceptions, therefore, all the work on strain selection so far has been directed toward superior streptomycin-producing strains. No best method seems to have been evolved, though three major

Formation of Other Antibiotics by S.griseus

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and streptomycin production is a most remarkable observation, and one well worth further study.

The third technique was announced almost simultaneously by Vanderlinde and Yegian (11) and by Iverson and Waksman (12). It follows upon the discovery of a streptomycin-dependent meningococcus by Miller and Bohnhoff (13). Vanderlinde and Yegian used streptomycin-dependent strains of *E. coli* and *Ps. aeruginosa* as well as sensitive and resistant, but not dependent, strains of *E. coli* (figs. 5, 6, 7).

Iverson and Waksman (12) used the same principle to develop a method for detecting streptomycin in broth. Growth of a streptomycin-dependent strain of *E. coli* produced turbidity proportional to the streptomycin con-

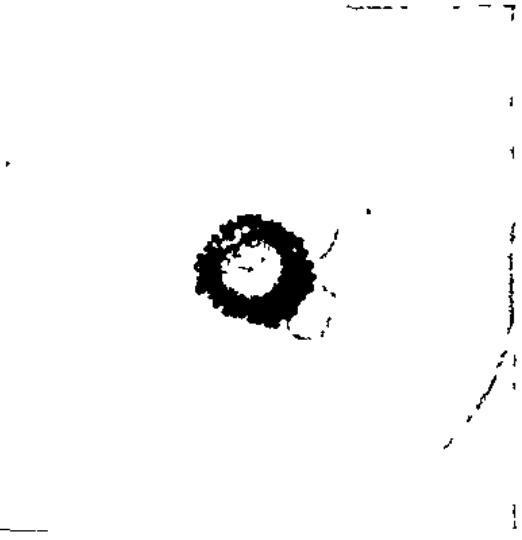

FIG 5. (
surroundin
inoculated
cete's growth (11).

centration over a suitable range. Crude or pure streptomycin could be used. Streptothricin-producing, grisein-producing, and nonstreptomycin-producing cultures did not support growth of the streptomycin-dependent *E coli*, hence would not appear as streptomycin-producing cultures in a screening program employing this technique. The use of antibiotic-dependent strains should find wide use in screening programs for other antibiotic-producing cultures.

Natural Variation and Selection

No extensive programs have been reported in which a diligent search has been carried out for superior strains among mutants arising from natural variation Waksman has shown repeatedly that streptomycin-producing strains of *S. griseus* produce mutants readily. A nonconidial mutant that

methods have been employed: (a) search for superior strains in nature, (b) examination of old strains after natural variation has taken place, and by various selective techniques, and (c) application of mutagenic agents to cultures of known potentialities, followed by screening for superior strains among the mutants formed.

Superior Strains from Nature

The brunt of the search for superior strains from natural sources has been borne by Waksman, Reilly and Johnstone (1). The fact that only three streptomycin-producing strains of *S. griseus* have been found by Waksman and only two by Carvajal (2) supports the view that although *S. griseus* is fairly easy to isolate, only a very few of all the strains isolated will be streptomycin producers. Carvajal isolated *S. griseus* many times from such sources as soil samples, river mud, insects, plant roots, air, foodstuff, animal excreta, water, decomposing plant material, and dust. No particular attention was drawn to the fact that both active strains were isolated from river mud. Waksman seems to have isolated all three of his strains from either soil or composts. Soil rich in organic matter appears to be the best source of active strains Unfortunately, neither Waksman nor Carvajal indicates how many cultures were screened for their particular number of successes.

The effort required to establish that a particular antibiotigenic *S. griseus* is producing streptomycin has naturally inspired a search for screening techniques that would allow the identification of streptomycin-producing strains on the original streak-plate The following three techniques have been developed:

The first employs addition of 100 μg of streptomycin to each milliliter of agar upon which isolations are to be made (1) All cultures sensitive to this concentration are inhibited, 80 per cent in one experiment The survivors will contain streptomycin-producing cultures (no. 3481 was isolated in this way) and other resistant cultures The method is very useful for preliminary screening of soil cultures and is equally adaptable to the search for other specific antibiotic producers.

The second technique employs the discovery (9) that streptomycin-producing strains of *S griseus* are sensitive to actinophage, whereas all other strains of *S griseus* tested are resistant. This technique has the advantage of extreme simplicity, but the disadvantage of missing any streptomycin producers that happen to be resistant to phage That such phage-resistant strains exist has already been shown (6, 10). The wild type of all streptomycin-producing strains of *S griseus* may be predominantly sensitive to phage, but this technique would not bring out that fact. Regardless of its utility or infallibility, this positive correlation between phage sensitivity

by mutagenic agents of new, superior penicillin-producing strains. In the first 4 years of streptomycin research, reports of similar accomplishments have been scanty. It is not readily apparent whether this is due to less effort by investigators or to an inherent inability of *S. griseus* to react to the mutagenic techniques that have been employed. No doubt, the rapid development of superior fermentation media has removed much of the stimulus for this work.

The most readily employable mutagenic techniques use radiant energy in the ultraviolet and x-ray regions. In a preliminary report (15), Stanley showed remarkable success with the use of ultraviolet. Details of parent strain, wavelength used, dosage, mutation rate, and strain stability were not reported. Until a more complete report is made, this work is difficult to evaluate. Similar success is not reported by Savage (10), using ultraviolet of 2,537 Å wavelength. Although the parent strain, an isolate of Waksman's no. 3496 culture, mutated with fair efficiency, the mutants were low in high-yielding strains, and only one, RM-241, in some 1,400 strains tested proved high yielding to a stable degree. Further irradiation of RM-241 with 2,537 Å ultraviolet was ineffective. Almost no mutations took place.

X-rays of three wavelengths were employed. All were found to be mutagenic. The longest of the three rays (1.539 Å) proved to have only one-fourth the mutagenic activity of either of the two shorter rays (0.710 Å and 0.210 Å). Doses up to 1,000,000 roentgens were employed to obtain mutation rates up to 50 per cent of morphological mutants and up to 40 per cent of physiological mutants, only the single characteristic of streptomycin production being scored. Two strains proved to be stable and superior to a marked degree, out of some 2,300 strains screened. One, RM-2451, was selected as superior. A remarkable instance of strain specificity for the medium employed in strain selection is shown in figures 8 and 9.

Waksman's original beef-extract-peptone medium was used to obtain the fermentation curves in figure 8. This was the medium used for the selection of strain M4, the isolate from Waksman's no. 3496 culture. The superiority of M4 over 3496 is apparent. Figure 9 shows the same four strains on a yeast-glucose-salts medium which was used for the selection of RM-2451. The four strains arrange themselves in the order in which they were selected one from the other. RM-2451, however, is a very inferior strain on Waksman's medium (fig. 8), producing almost undetectable amounts, 4–6 μg/ml, of streptomycin.

The extremely low yield of superior and stable mutants, 0.1 per cent, indicates the need for superior plate-screening methods such as that developed by Vanderlinde and Yegian for elimination of nonyielding and low-yielding strains. It is possible that the use of streptomycin-dependent strains in a plate-screening procedure will provide an answer to this need.

produces no streptomycin, and a pink mutant that produces another antibiotic have appeared repeatedly. Those mutants or variants that produce streptomycin have invariably produced less streptomycin than the parent culture. The technique of adding 50 μg/ml of streptomycin to the agar proved helpful in eliminating low-yielding strains (14) but did not permit the isolation of any superior strains. Two of the high-yielding strains so isolated from submerged culture were found to have lost most of the ability of the original culture to produce streptomycin in surface culture. Carvajal (2) showed photographs of the extensive sectoring of *S. griscus* colonies

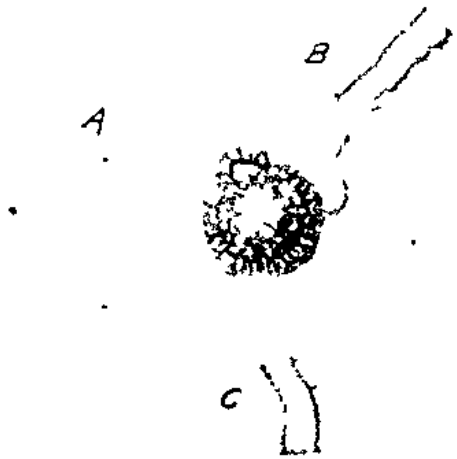

FIG. 6

FIG. 7

FIG 6. Three strains of *E. coli* on a plate containing a central growth of a streptomycin-producing strain of *S griseus*. A, a strain of streptomycin-dependent *E. coli* showing growth only in the portion of the streak near the actinomycete. B, a

FIG. 7. Spread of growth of streptomycin-dependent *Ps aeruginosa* about a growth of a streptomycin-producing strain of *S. griseus*. The bacteria were inoculated at the tip of the *S. griseus* streak (11)

which occurs after 1 to 3 months' growth. He reported variation in rate and extent of spore production, color, pigment production, odor, and colony appearance. Strains showed preferences for specific media for optimal spore production and for the production of most of the above characteristics. No information has been reported on the influence of these variations upon streptomycin yields. The changes noted were observed on both active and inactive cultures.

USE OF MUTAGENIC AGENTS

One of the most exciting developments in penicillin research of the last few years has been the isolation from natural sources and the development

in concentrations in the culture medium rise and then slowly fall indicates that the concentration at any time is the resultant of a process of formation and one or more competing processes of destruction or inactivation. Henry *et al.* (17) have shown that culture media components may inactivate as much as 75 per cent of added streptomycin. In addition, such compounds as hydroxylamine and cysteine have been shown to be able to inactivate streptomycin.

Any mutation that would destroy or suppress a reaction competing with the streptomycin synthesis for substrate, or any mutation that would destroy or suppress the synthesis of any streptomycin-inactivating metabolite, would cause the new strain to synthesize more streptomycin if the streptomycin-synthesizing mechanism was unharmed.

The results reported by Whiffen, if substantiated, would indicate that the syntheses of actidione and streptomycin may be competing for substrate.

So far there have been no reports of successful use of the nitrogen mustards as mutagenic agents in streptomycin fermentation. Their recent use in effecting mutations in *E. coli* (18) indicates the possibility of their being used successfully with the *Streptomyces.*

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Mutant strains from these x-ray studies have been used to investigate other strain relationships. Gottlieb and Anderson (16) compared strain RM-1067, which produced no detectable amount of streptomycin, with a high-yielding strain and with an intermediate-yielding strain. They found no correlation between streptomycin production and respiration rate of *S. griseus*. Of the three strains tested, those producing the highest and the lowest quantities of streptomycin each had a QO_2 almost three times that of the strain producing an intermediate amount of streptomycin.

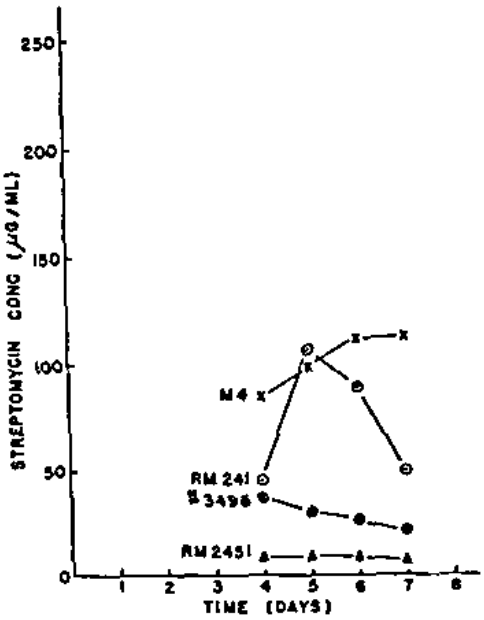

FIG. 8

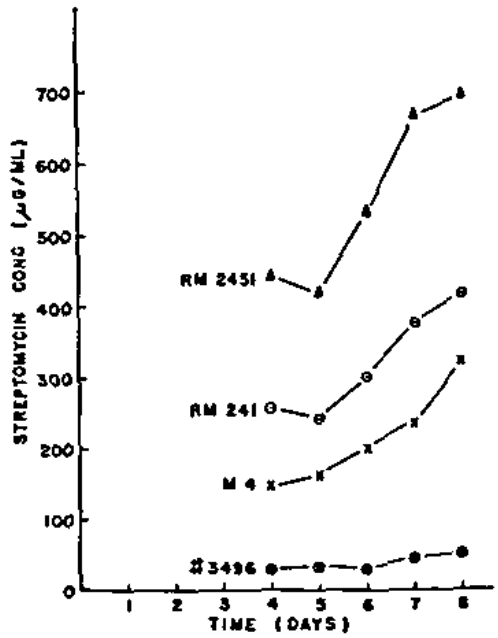

FIG. 9

FIG 8. Fermentation curves of R C.C. 3496 and three strains derived from R.C C· 3496 The medium is Waksman's original beef-extract-peptone-glucose-sodium chloride medium for the production of streptomycin.

FIG 9 Fermentation curves of the same strains in a yeast-glucose-salts medium M4 was selected from 3496 RM-241 is an ultraviolet-induced mutant of M4. RM-2451 is an x-ray mutant of RM-241

Whiffen (3) has analyzed 144 isolations of *S. griseus*, obtained after x-ray irradiation of RM-241. She found that mutation can influence streptomycin and actidione production independently, except that the yields of both cannot be enhanced together Some of the mutants have higher streptomycin yields and lower actidione yields than the parent culture. In others, the situation is reversed. In some, the yields of streptomycin and actidione are both significantly lower than those for the parent. In no instance were mutants found to produce more streptomycin and actidione than the parent.

These results shed some light on the mechanism by which a strain is converted to a higher-yielding strain. It is conceivable that this might be accomplished by any one of several ways The very fact that streptomy-

kg. The ultimate level of production and utilization of streptomycin has not been reached, and to predict what level may be attained is difficult.

At the outset of this discussion on the production and isolation of streptomycin, it should be understood that it is not possible to include many of the significant details of the existing industrial processes. Naturally, the published operational details for the production of this relatively new and highly important drug are meager and will remain so for a number of years. Although the earliest described industrial processes (3, 4) have undergone numerous changes, the basic steps remain essentially unchanged, and consist of fermentation, recovery, purification, and finishing. Each of these steps is described in detail in the following pages.

Fermentation

The production of streptomycin broth is a pure culture aerobic fermentation of *S. griseus*, carried out under aseptic conditions. The first description of the fermentation indicated that both a shallow-layer stationary method and an aerated submerged procedure were satisfactory (5). The stationary method was used at the outset but was rapidly displaced by the more economical submerged growth method when the required facilities became available. Since the stationary procedure is of no industrial consequence, and since adequate details of a unit for the production of streptomycin broth by surface culture in pint milk bottles are described in a recent report (6), the present discussion is limited to the submerged process.

All cultures now used in the production of streptomycin are believed to have originated from one of the original isolations of *S. griseus* No. 18-16. Because this organism is unstable and its variants differ in their productivity (7), the care and selection of the strain are highly important. Higher-yielding strains have been selected from natural variants and from mutant strains produced by controlled exposure of cultures to ultraviolet light irradiation. Some of the selected mutants in the usual large-scale production media are capable of producing broths in the laboratory with potencies of more than 900 μg/ml, a level significantly higher than that obtained from the parent culture (8).

Although operating conditions and fermentor design have played an important role in the maintenance of large-scale maximum productivity, the more important increases in the level of production have been brought about by microbiologists through use of improved media and selection of high-yielding strains (51).

Streptomycin fermentation is subject to severe bacteriophage attack, and for successful production the development of phage-resistant strains was

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CHAPTER 4

PRODUCTION AND ISOLATION OF STREPTOMYCIN

During the last 10 years, production of the now established antibiotics, penicillin and streptomycin, has grown from the shake-flask and test tube scale into an important segment of the chemical industry. This rapid growth is truly a tribute to the laboratory, technological, and clinical scientists who have taken part in the development.

The production of antibiotics begins with fermentation, a tool previously of little importance to manufacturers of medicinals. Although fermentation is one of the oldest chemical processes practiced and has considerable economic significance due to widespread use in industry, it must still be regarded as an art rather than as a science. The manufacturers of medicinals had to adapt this process, drawing upon the experiences from existing industrial fermentation processes and discovering new principles that resulted from the combined efforts of the microbiologist, the engineer, and the chemist. Industrial progress in the field of antibiotic fermentation has resulted from the increased recognition and expansion of the engineering phases of fermentation, from the application of the physical-chemical approach to the solution of fermentation problems, and from the development of high-yielding mutant strains by new microbiological techniques.

Notwithstanding the countless problems that required solution, the production of the antibiotics is now a well-developed industry. The industry has kept pace with the increasing demands and is satisfying the needs of the physician in the combat of disease and in the prolongation of life. The combined sales of penicillin and streptomycin in the United States during 1947 totaled $111,950,000 and equaled half the sales of all the synthetic drugs sold during this period (1).

Production of significant quantities of streptomycin began in August 1946, and during that year about 1,000 kg were produced. The output for 1947 was 10,000 kg (2), and the production for 1948 approximated 36,000

[1] The author is indebted to Mr. F. W MacMullen for his valuable assistance in the preparation of this manuscript.

lization of the medium has been successfully demonstrated and may have some economic advantage. To maintain the aseptic conditions necessary for this fermentation, all equipment must be designed to avoid pockets, which may serve as breeding places for contaminating organisms. All piping and equipment must be connected to high-pressure steam lines for sterilization. Sterility of equipment and medium is essential for satisfactory production.

The composition of the medium has a marked influence on the production of streptomycin. The original medium recommended by Waksman consists of an aqueous solution of 1 per cent glucose, 0.5 per cent peptone, 0.3 per cent meat extract, and 0.5 per cent sodium chloride (5). Several successful substitutions of less costly ingredients, however, have been made for glucose, peptone, and meat extract. Starch can be substitute for glucose; unhydrolyzed and hydrolyzed casein, various tryptones, or sodium nitrate can replace peptone (11); and corn steep liquor (11) or soybean meal (12) is reported to be approximately as effective as meat extract.

One of the difficulties encountered during the fermentation is the tendency of the fermenting mash to foam. For this reason the amount of air and agitation must be kept at the proper balance, since too much air causes excessive foaming while too little inhibits bacterial growth. For the control of foaming a surface-activating substance may be used, which must be sterilized before addition to the fermentor. Such a foam inhibitor must be nontoxic to the organism and must not interfere with the subsequent steps of recovery and purification.

The fermentation cycle in a fermentor varies from one to several days depending upon the culture, the medium, and the conditions employed. Selection of the actual cycle time depends upon the economic evaluation of productivity per gallon per hour. The streptomycin content of the commercial broths has not been reported, although laboratory yields ranging from 150–900 μg/ml in production-type medium have been published.

Recovery of Streptomycin from Fermentation Liquors

Streptomycin is a highly polar organic base, which may be considered as a derivative of a carbohydrate. The structural formula indicates that streptomycin is a complex molecule containing a large number of hydrophilic and functional groups The two guanido groups in the streptidine portion of the molecule and the methylamino group in the N-methylglucosamine moiety are responsible for the strong basicity.

Streptomycin

Streptomycin, as the free base or as a salt of an inorganic acid, is extremely soluble in water. Because of the high alkalinity and the instability

necessary. This has been accomplished by suitable culturing methods similar to those used in the acetone-butanol fermentation (9).

A fairly complete description of the fermentation procedure employed at Merck & Co., Inc., has been reported by R. W. Porter (3). A digest of this report together with information from other sources is now presented.

An agar slant is inoculated with a small quantity of the culture and is incubated to sporulation under carefully controlled conditions. The spores are suspended in distilled water, and the suspension is divided into three parts, each of which is transferred to a shake flask plugged with cotton and containing about 300 cc of nutrient solution. These flasks are then placed on a shaking machine in a temperature-controlled room. This is the first stage of submerged fermentation.

To build up the size of the inoculum for the large production fermentors, the laboratory culture has to be transferred through a series of increasingly larger fermentors. Each vessel in this series is designed so that it will provide the required quantity of growing organisms or inoculant for efficient utilization of the next larger fermentor in the series.

The laboratory culture is transferred aseptically to the first of the series of fermentors. Each stage in the fermentation follows its own particular time schedule. From the first fermentor to each succeeding fermentor, the inoculum is transferred aseptically until finally it reaches the large fermentors.

Fermentation conditions are generally the same throughout the series of fermentors. The temperature is carefully controlled around 25°C by circulating temperature-controlled water through jackets or coils built into the fermentor. Aeration is accomplished by forcing through the fermentation liquid air that has been sterilized by passage through filters to remove air-borne organisms. Agitators in the fermentors help promote dispersion of air

Several types of equipment for aeration have been used, these include the open pipe sparger, the sintered glass aerator, and horizontal pipes containing small openings along their lengths The air is introduced at the bottom of the fermentor and the action of the agitator affects even distribution throughout the medium For maximum yield of streptomycin good contact between liquid and air is essential, since the oxygen demand of submerged cultures of *S griseus* is high (10).

The pH of the medium is controlled At the beginning of the fermentation the medium is neutral but may rise to pH 8 to 8 5 as the fermentation progresses

Sterilization of the medium is accomplished by heating the medium in the fermentor to 120°C and maintaining this temperature for one-half hour before cooling to the fermentation temperature of 25°C. Continuous steri-

practically pure chemical compounds. Several reports of industrial isolation procedures have appeared (3, 4, 16, 17).

Filtration of harvested broth

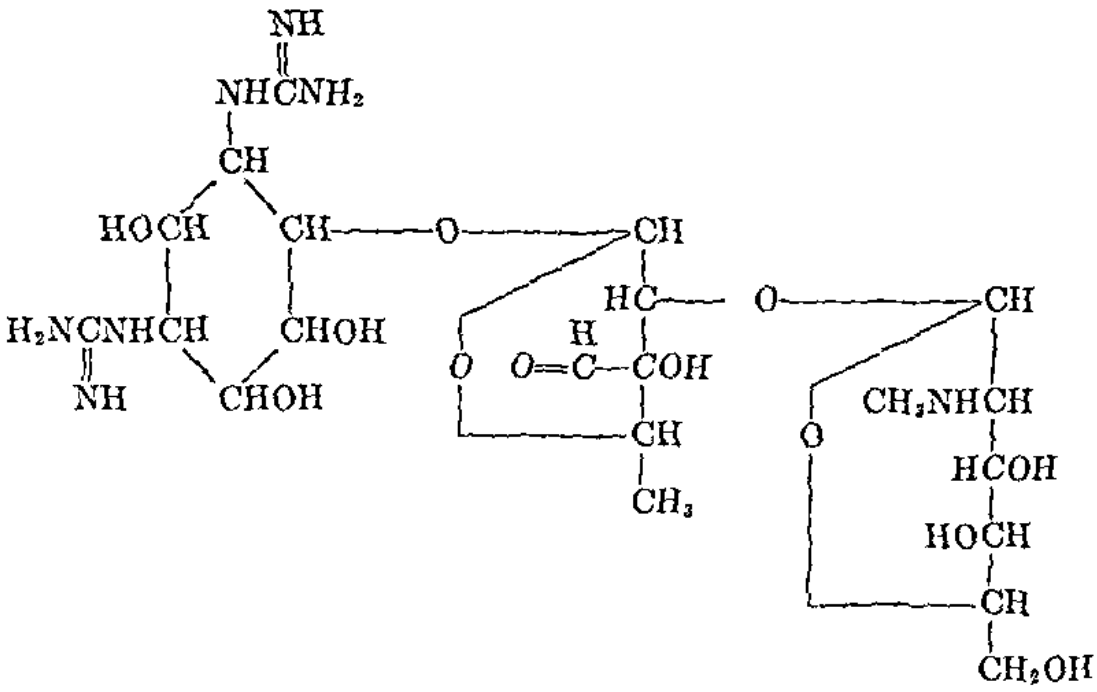
The first step in the isolation of streptomycin from harvested broth is the separation of the insoluble solids composed primarily of mycelium, which is extremely fine and gelatinous in character. Filtration is difficult, and significant losses are experienced if the filtration and washing of the solids are not carried out properly. Filtration, even on a laboratory scale, requires a filter-aid. In large-scale operation the most successful filtration procedure consists in the use of a continuous pressure filter. With the appropriate use of a filter-aid, and by making compensating variations in the operating conditions (4), this type of unit can be used in a continuous manner, in spite of great variations in the filterability of individual harvested batches. A filter-aid is used both as a precoat for the filter and as an admixture in the broth. Selection of the filter-aid requires care, since many of the commercially available filter-aids adsorb streptomycin.

Treatment of the whole broth before filtration has been recommended for facilitating filtration and for removal of impurities, including pigments, which interfere with the efficiency of the subsequent isolation steps (13, 18, 19). In this procedure the whole broth is acidified with hydrochloric or sulfuric acid to pH 2 (13, 19) or to pH 4 (18), stirred with charcoal (Norit A or Nuchar C-190-H), and the mixture filtered. At this low pH streptomycin is not adsorbed and is stable for considerable time (20). A significant quantity of the impurities, however, is adsorbed by the charcoal. After the pretreatment operation, the clarified, decolorized filtrate is neutralized with caustic to pH 7.0 to 7.5 and is ready for the adsorption step.

Adsorption of streptomycin from filtered broth

The literature on the adsorption of streptomycin from water solutions with adsorbents other than charcoal is sparse. A large number of adsorbents undoubtedly have been investigated in the search for a more specific adsorbent. Fuller's earth adsorbs streptomycin readily, but elution of the antibiotic from the adsorbent presents difficulties (21). Other adsorbents of little interest in the isolation of streptomycin from broth are alumina, silica gel, inulin, diatomaceous earth, cellulose products, magnesium silicate, and magnesium phosphate (21).

Cation exchangers, such as the zeolites, have been investigated by LePage and Campbell (18) in an attempt to find a more specific agent than charcoal for the isolation of streptomycin from harvested broth. Certain of these agents were reported capable of adsorbing streptomycin, but they showed no advantageous specificity. The zeolites such as Decalso and Permutit,



of the free base, little is known about its solubility in organic solvents. Streptomycin salts of inorganic acids are insoluble in nearly all organic solvents. Attempts to extract streptomycin from an aqueous solution over a pH range of 2 to 9 with butanol and with other common water-immiscible solvents were unsuccessful (13). Streptomycin salts of organic acids do show solubility in organic solvents. Titus and Fried (14) reported that streptomycin salts of organic sulfonic acids, such as *p*-toluene sulfonic acid, can be extracted from water solutions by butanol.

As evident from this discussion, streptomycin does not lend itself readily to a solvent-extraction process of isolation from harvested broth. Recovery on an adsorbent, such as charcoal, instead, is the basic concentration step in all the practical, published processes. This concentration step and the subsequent acid elution of the adsorbate were first reported by Shatz, Bugie, and Waksman (5) and were used previously for the isolation of streptothricin (15).

Since carbon is a relatively nonspecific adsorbent, a great many other substances, some of which are toxic, are isolated along with streptomycin. This fact makes necessary further fractionation before the drug is safe for therapeutic use. Improvements in the purification steps have been developed and applied successfully to production. In contrast with the variable quality concentrates of streptomycin available at the outset of large-scale production, a number of the commercial products presently available are

advantageous, since the carbon requirements for large-scale operations are very large.

Elution and recovery of crude streptomycin

As mentioned previously, carbon adsorbates contain considerable quantities of impurities, and therefore methods for the partial removal of these impurities from the adsorbate prior to elution had to be developed. The common practice now is to wash the carbon adsorbate with solvents before the elution step. Washing the adsorbate with copious quantities of water (17, 18, 19) or with aqueous methanol or ethanol (3, 13) does not elute streptomycin but does remove some impurities that otherwise would contaminate the product.

Elution of carbon adsorbates is carried out in all processes with dilute acids. Generally, sufficient acid is employed to maintain a pH 1.5–2.5 for the elution slurry (17, 19). Alcoholic hydrochloric acid solution was the first successful eluting agent used (5), and several modifications of this eluting solution have found application in subsequently reported processes. For large-scale operation a two-stage countercurrent elution procedure has been reported in order to maintain low elute volumes Such elutes are neutralized and concentrated under reduced pressure, and the residue is diluted with methanol and then acetone (17). The precipitated crude product is collected and dried. The neutralization of the elute can be carried out with caustic soda, in which case a filtration of inorganic salts is required before the acetone precipitation. In general, the eluate volumes are 0.1 to 0.05 of the volume of the whole broth.

A convenient modified eluate is 0.1 *N* methanolic hydrogen chloride (13), since crude streptomycin hydrochloride can be isolated from the eluate by addition of dry ether. Use of 60 per cent acetone-water containing hydrochloric acid for elution has been reported (19). The eluate in this instance is neutralized with caustic soda, concentrated under reduced pressure, and the product is precipitated by addition of sufficient acetone to bring the acetone concentration to 80 per cent. This procedure has the advantage of using only one solvent, thus avoiding the recovery and fractionation of large volumes of mixed solvents.

The elution of carbon adsorbates with dilute sulfuric acid and the isolation of crude streptomycin as the sulfate have been found attractive (19). An eluting solution of 5 to 10 per cent acetone-water containing sufficient sulfuric acid to maintain pH 2.5 during elution is effective. Addition of acetone until its concentration is 75 per cent precipitates streptomycin sulfate in good yields This procedure avoids the need for neutralization and concentration

A dilute aqueous solution of phosphoric acid has been employed as the

however, have been applied with success to the extraction of streptothricin from broth (22). The elution of the antibiotic from the exchanger is accomplished by treatment of the complex with saturated solutions of sodium chloride Since the antibiotic is mixed with a large quantity of salt, a fractionation using methanol is required for separation of the two.

Synthetic organic sulfonic acid-type cation-exchange resins have been tried unsuccessfully for the isolation of streptomycin (23). Although such resins adsorb streptomycin by ion exchange, the resin-antibiotic salts are so stable that most reagents, such as inorganic acids, inorganic salts, organic acids, morganic or organic bases, employed to elute or desorb the antibiotic are required in such quantities or in such strength that the streptomycin is either destroyed or greatly contaminated with the eluting agent. The strong acidity of the sulfonic acid exchangers precludes high specificity because of the presence of a considerable quantity of nitrogen bases in the broth.

The adsorption of streptomycin from filtered broth is readily accomplished by addition of activated carbon and agitation of the mixture, followed by filtration. The pH of the filtered broth affects the adsorption efficiency. With all activated carbons, pH 6–8 is optimal. Pigment adsorption increases, whereas the total solids and streptomycin adsorption decrease below pH 6; and at pH 2 no significant adsorption of streptomycin occurs (17). The quantity of carbon required for efficient adsorption depends upon the nature of the carbon and upon the quality of the broth. With solutions of pure streptomycin hydrochloride, carbon capacities of 80,000 μg/gm, depending upon the nature of the carbon, have been obtained The capacity of activated carbon is lower when it is applied to filtered broth. Capacities of 10,000 to 20,000 μg gm are observed when broth of 300–400 μg ml is used. The selection of a satisfactory carbon and the optimal requirement of carbon must be established for each type of broth

Large-scale adsorption of streptomycin is generally carried out on a continuous basis (3, 4) The carbon is introduced into the clear liquor automatically at a predetermined rate and thoroughly mixed by passing the slurry through tanks with efficient agitators before the mixture is filtered. The addition of carbon must be carefully controlled, since insufficient carbon results in incomplete adsorption, and excess carbon reduces elution yields. The rich carbon is separated by the use of a continuous filter.

Use of large-scale static carbon beds for adsorption and elution has been reported (17). No quantitative comparison of the performance of charcoal suspensions versus column adsorption and elution was recorded. The observation has been made that static carbon beds could be used for successive adsorptions and elutions without impaired efficiency (17). This feature is

advantageous, since the carbon requirements for large-scale operations are very large.

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eluting solution (17). The introduction of phosphates, however, is disadvantageous, because troublesome, insoluble phosphates are formed when the eluate is neutralized with caustic. An organic acid such as formic acid (24) in 50 per cent methanol-water is also an effective eluting agent. A crude precipitate of streptomycin formate is obtained by concentration of the eluate to a small volume, dilution of the residue with methanol, followed by addition of acetone.

Two processes (17, 18) employ a two-stage carbon adsorption and elution procedure. In these cases the first eluate or the crude solid obtained from the eluate is readsorbed on charcoal and eluted again. Fractionation of the first crude product is accomplished in one instance by carrying out the adsorption portionwise first at pH 4 and then at pH 6.0. The second adsorption and elution bring about a significant purification.

Inasmuch as the potency and the quality of the crude streptomycin are related to the nature of the harvested broth, evaluation of the relative efficiencies of the known concentration procedures is difficult. Crude products ranging in potencies[2] from 200 to 600 μg/mg in yields of 30 to 65 per cent (based on whole broth) have been reported.

Purification of Streptomycin Concentrates

In the early pharmacological and clinical studies of streptomycin, toxic reactions frequently were observed. Variation in the pharmacological reactions also was noted, which could be attributed only to the low quality and variability of the available streptomycin. There was recognition almost from the outset that purification was mandatory, if the drug was to be released for widespread use by the medical profession. Among the toxic impurities that needed elimination or reduction were those causing pyrogenic response, acute toxicity in mice following intravenous injection, histamine-like reactions, neurotoxic manifestations, and irritation and pain reactions after injection.

A number of different methods of purifying crude streptomycin are now known. These methods will now be discussed.

Chromatography

The application of chromatography to the purification of streptomycin has been well summarized by Peck (25) in a paper presented before the New York Academy of Sciences. Chromatography has proved to be an invaluable tool in the purification of streptomycin and has been used in nearly every known process for the preparation of an essentially pure product.

Two adsorbents, alumina and activated carbon (26), have been employed

[2] ... hydrochloride has a potency of 830 μg/mg.

for column chromatography, although the former has been studied more intensively. The procedure employed in general is to percolate a solution of streptomycin hydrochloride in 80 per cent methanol-water at pH 5–6 through a vertical column packed with a pretreated alumina. As the streptomycin passes into the column, 80 per cent methanol-water is fed continuously into the column until the eluate shows the presence of only a negligible quantity of streptomycin. Since streptomycin is colorless and is similar in properties to many of its impurities, visual observation of the chromatographic performance is not possible. The location of the purified eluate fractions was determined at first by microbial assays; but later Carter, Clark, Dickman, Leo, Snell and Strong (13), in the first literature description of the chromatographic purification procedure, employed the Sakaguchi test for guanido groups and a chloride test to locate the active eluate cuts. More specific color reactions and rapid chemical assays are now available (27, 28), which facilitate the operation of the alumina columns.

According to the procedure of Carter and his associates, the alumina was washed first with dilute sulfuric acid and then with water until the washings were free from sulfate, and dried. The weight ratio of adsorbent to crude streptomycin employed for chromatography was 25:1 to 50:1. In following the progress of chromatography a microbiologically inactive filtrate fraction giving a positive Sakaguchi test was observed, which was followed by a fraction giving a negative Sakaguchi test Subsequent fractions gave positive to strongly positive tests, paralleling the microbiological evaluation. A small amount of active material remained on the column and was removed only by increasing the water content of the developing solution. The later eluates contained sulfate but no chloride ions, indicating an exchange of anions. Since streptomycin sulfate is less soluble in methanol-water than the hydrochloride, the behavior is understandable. The most active fractions of the eluate were concentrated to remove the methanol and lyophylized to a white product, which according to microbial assays was almost pure

The use of alumina washed with hydrochloric acid instead of sulfuric acid has also been reported (19). In this case no retention of activity was noted, as is experienced with the alumina washed with sulfuric acid.

Alumina chromatography of crude streptomycin hydrochloride has been applied successfully to the large-scale production of streptomycin (29). According to this report the procedure is similar to those already discussed. The large columns are filled with 100–200-mesh alumina and are operated under a pressure of 60 pounds per square inch. The performance of the chromatography is followed by spot analysis of the eluate cuts.

The purification efficiency of alumina chromatography is to some extent

dependent upon the potency of the crude streptomycin hydrochloride. With very low potency crudes, a preliminary purification is essential. Picric acid treatment of a low quality crude prior to chromatography (26) illustrates this point.

An examination made by Peck (25) of all the available quantitative data indicates that during alumina chromatography streptomycin passes through the column at a faster rate than the impurities. This report indicates that streptomycin is about the least strongly adsorbed component of crude concentrates. The addition of alumina in portions to a methanolic solution of crude streptomycin hydrochloride removes a significant amount of the impurities, with a concomitant increase in purity of the product remaining in solution (26). The development of this principle into a practical process was reported by Mueller (30) The method does not require columns and employs a methanol solution of crude streptomycin phosphate-hydrochloride. Addition of several portions of alumina and separation and elution of each portion gave a favorable fractionation of the crude. By this process preparations of 200-300 μg/mg could be increased to 600-650 μg/mg.

Purified streptomycin is obtained from the eluates by concentration to small volume and lyophylization of the aqueous residue or by application of the solvent precipitation procedures discussed previously.

The chromatography of inorganic salts other than the hydrochloride of streptomycin and the sulfate has not received much attention, primarily because of the low solubility of these salts in methanol-water. Colored salts of streptomycin with organic acids have been subjected to chromatography (31, 32), but they did not exhibit any advantage over the hydrochloride

Other purification methods

Since streptomycin is known to be an organic base, it is only natural that a number of the known amine precipitants should have been tested for usefulness in the purification of this compound. The experience gained in the purification of streptothricin by the use of organic acids (24, 31, 32) gave added impetus to this approach

A number of crystalline water-insoluble salts of streptomycin were prepared during the early investigations. Not only were they useful in the isolation of pure streptomycin but they also facilitated establishment of the empirical formula of the antibiotic Among these salts were the reineckate (31, 33), the helianthate (26, 32), and the *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonate (26, 32) The last two derivatives require Methyl Orange and Orange II respectively for their preparation. These derivatives were obtained in crystalline form only when the streptomycin concentrate used was at least 50 per cent pure, and consequently they were prepared after the

crude streptomycin had been chromatographed. When concentrates of low purity were used for the preparation of these salts, mixtures were obtained that were difficult to purify. The crystalline derivatives were readily converted into essentially pure streptomycin hydrochloride, hydrobromide, or sulfate by treatment with the appropriate inorganic acid. The hydrochloride, for example, may be prepared from streptomycin helianthate by stirring a mixture of the latter and a solution of hydrochloric acid-methanol (ratio 1:26 by volume) until the helianthate has been consumed. The insoluble Methyl Orange is separated by filtration through a layer of decolorizing carbon, and the filtrate is diluted with a copious quantity of acetone. The precipitated streptomycin hydrochloride is obtained in almost quantitative yield, and after drying is analytically pure (26).

Streptomycin is precipitated by picric acid (26, 31) and phosphotungstic acid (31), but no reports have appeared describing whether these salts have been pure or whether they have been obtained in crystalline form. Both salts, however, have been used effectively at some step in the preparation of pure streptomycin. Picric acid has not received serious consideration for large-scale use because of the possible hazards involved.

Streptomycin forms precipitates with tannic acid and with a number of commercially available dyes containing sulfonic acid and carboxyl groups (34). Among these dyes are Indigotine Certified, Solantine Yellow, Erie Fast Orange G. G., Niagara Blue, Azo Bordeaux, and Erie Fast Brown. These dyes in general are of limited value in the purification of crude streptomycin because of their lack of selectivity.

The preparation of a crystalline form of streptomycin suitable for therapeutic use is of considerable importance, since crystallization is generally a means of achieving a high degree of purity and generally ensures constancy of chemical composition. The conversion of a crystalline salt of streptomycin that is not pharmaceutically satisfactory (such as the helianthate) into a clinically acceptable salt does not, however, necessarily offer the same assurance, since the chemical agents required for the conversion may in some way affect the streptomycin molecule.

Peck, Brink, Kuehl, Flynn, Walti and Folkers (35) observed that streptomycin hydrochloride and calcium chloride form a crystalline double salt. This observation was of special importance, since this double salt was the first crystalline form of streptomycin suitable for parenteral use. The ultimate purity of this complex could be ascertained with certainty by solubility analyses, and consequently the pharmacology of streptomycin itself could be studied with assurance. As the double salt can be recrystallized readily, a product with constant chemical, biological, and physical properties can be obtained even from crude streptomycin of questionable character.

Crystalline streptomycin trihydrochloride calcium chloride double salt, also referred to as *streptomycin calcium chloride complex*, may be prepared by mixing methanolic solutions of streptomycin hydrochloride and calcium chloride. By proper adjustment of volumes, the complex crystallizes from the solution in high yield as elongated needles.

Streptomycin hydrochloride of 40–50 per cent purity is suitable for preparation of the complex. Crude concentrates of lower purity can be used, but

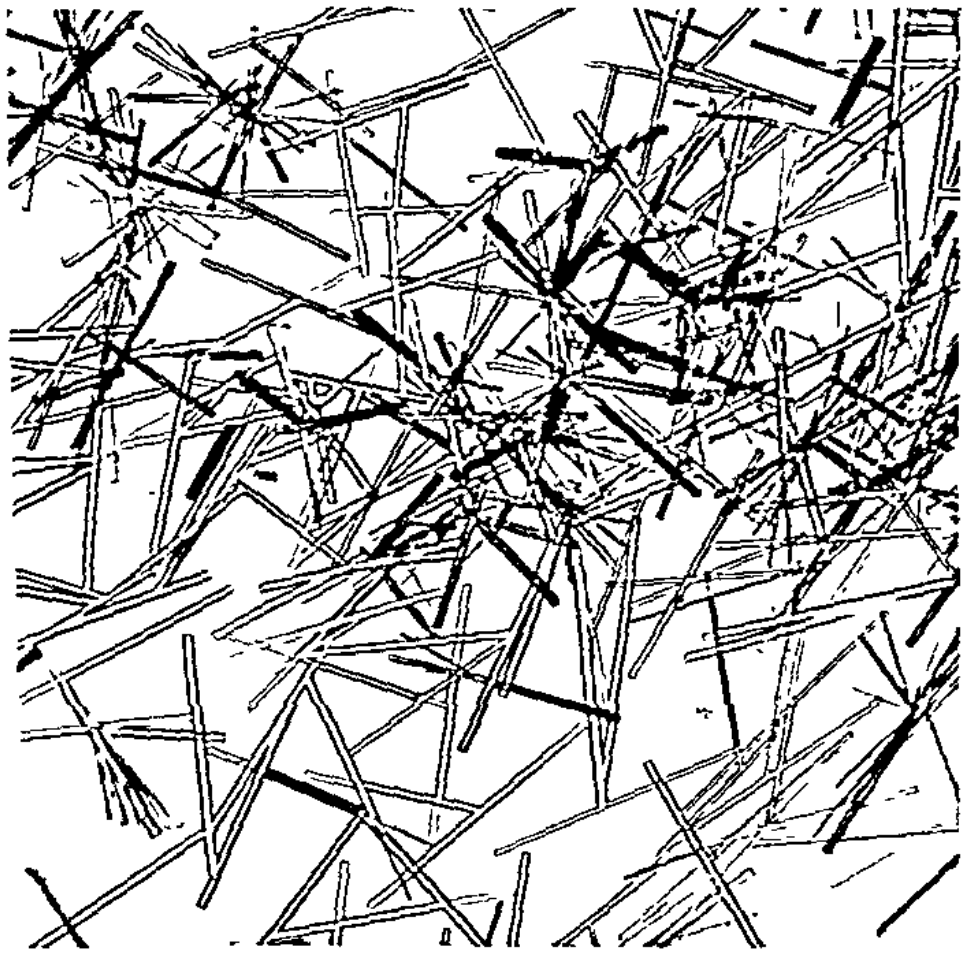

FIG 10 Crystalline streptomycin trihydrochloride-calcium chloride double salt (Courtesy of Merck & Co.)

the yields of recrystallized complex are likely to be lower The property of forming a crystalline double salt with calcium chloride fortunately has proved to be specific for streptomycin and is not shared by the impurities likely to be present in crude concentrates Samples of concentrate known to contain highly toxic impurities, pyrogens or blood-pressure-depressing substances, are rendered suitable for therapy by conversion into the crystalline double salt. Since neither streptothricin nor mannosidostreptomycin

(streptomycin B) (36) forms a crystalline complex with calcium chloride, under the optimum conditions for the formation of the streptomycin complex, these possible contaminants are lost in the mother liquor, especially if the crystallization conditions are properly controlled (37).

The preparation of streptomycin calcium chloride complex is being carried out on a large scale (16) in essentially the manner just described. The complex is produced for use as such and for the preparation of other salts of streptomycin, such as the sulfate and the hydrochloride. The preparation of crystalline streptomycin trihydrochloride has been reported recently (38). The crystalline salt was obtained from methanol as monoclinic prisms containing two molecules of water. A crystalline form of streptomycin trihydrochloride containing one molecule of methanol of crystallization was obtained from a solution of the salt in a mixture of methanol and acetone (39). The crystalline salt, isolated as fine needles having limited solubility in methanol (1 gm in 25 ml), loses its methanol and crystallinity when heated at 56°C *in vacuo*. No report of the crystallization of streptomycin sulfate has thus far appeared. This salt of streptomycin and the hydrochloride and the calcium chloride complex constitute the salts of streptomycin now in use by the medical profession.

Conversion of one salt of streptomycin into another is accomplished by the usual methods of metathesis. Thus, streptomycin hydrochloride was prepared from the sulfate by treatment of the latter with a stoichiometric amount of calcium chloride (19). A more general method employs synthetic anion-exchange resins (23), which facilitate the preparation of salts difficult to prepare by netathesis

Stability of Streptomycin

Knowledge of the stability of streptomycin is important with respect to the processing steps and to the storage of the final product. As previously indicated, streptomycin solutions are stable in moderately acid and alkaline solutions (17, 20). The maximum stability of culture filtrates and of aqueous and methanol solutions of streptomycin hydrochloride is in the range of pH 3.0 to 6.0 at temperatures below 25°C. Solutions of commercial streptomycin sulfate (100μg/ml) at pH 3 to 7 are stable for at least 60 days' storage at 7° and 25°C. At higher temperatures, inactivation occurs at a significant rate; at 95°C about 50 per cent of the streptomycin is destroyed in 4.5 hours. The stability data presented by Regna, Wassellek and Solomons (20) is given in table 6, in which the half life of purified streptomycin under conditions of pH and temperature is indicated.

Commercially available streptomycin salts containing less than 3 per cent moisture are stable when stored at room temperature over long periods. Very little, if any, inactivation was observed when samples were held at

50°C for 10 weeks. Recognition of the stability of streptomycin salts has resulted in the extension of the expiration date for the sale of commercial streptomycin salts from 12 months to 18 months from the time of packaging. The specifications of the Food and Drug Administration in 1945 required that the drug be refrigerated at 15°C. The refrigeration requirement was dropped in February 1948, when the expiration date was extended.

TABLE 6

Half life of pure streptomycin under different conditions of pH and temperature (20)

pH	HALF LIFE AT			
	7°C	28°C	50°C	95°C
	hours	*hours*	*hours*	*hours*
0.8	1200	110	8	
1.7	Stable	1500	90	
2.7	Stable	Stable	900	
5.5	Stable	Stable	4600	37
7.0	Stable	Stable		
8.6	Stable	1100	50	
9.5	3000	300	28	
11.2		16		

Compounds Related to Streptomycin

Mannosidostreptomycin

Fried and Titus (40), during a study of the alumina chromatography of streptomycin concentrates, obtained in addition to active fractions of high streptomycin content a number of less active, more firmly adsorbed fractions having a microbial potency of 150 to 200 μg/mg. The behavior of these fractions toward a modified Craig countercurrent distribution technique (14) and toward alkali suggested that they contained a new antibiotically active base closely related to streptomycin. The isolation of this new substance, named by the authors *streptomycin B*, was accomplished by treatment of chromatographic fractions with reinecke salt, whereby a crystalline trireineckate was obtained. After several recrystallizations of the latter from warm water, the reineckate was converted into streptomycin B sulfate by reaction with silver sulfate (36). Structural studies by Fried and Stavely (42) established that streptomycin B contains the structural elements of streptomycin joined glycosidically to D-mannose. This latter observation gave rise to the name *mannosidostreptomycin.*

Streptomycin and mannosidostreptomycin differ in their antibiotic microbial spectrum (43). Against *Kl. pneumoniae,* streptomycin is about 3.7 times as active as mannosidostreptomycin, and against *B. subtilis* the ratio

is about 5 to 1. The two compounds form approximately the same quantity of maltol on treatment with alkali. By comparing the maltol color assay (28) with the microbial assay (*B. subtilis*) it is possible to calculate with some degree of accuracy the quantity of mannosidostreptomycin in streptomycin. The quantitative estimation of the two in a mixture can be made by the modified Craig countercurrent distribution technique (36, 14). A colorimetric assay for mannosidostreptomycin in mixtures with streptomycin, which depends upon a color reaction of mannose, was published recently (44).

Streptomycin remains the drug of choice, since little is known about the pharmacology of mannosidostreptomycin. At present the latter is generally separated from streptomycin during the purification steps, but this practice represents an economic loss. Control of the fermentation process, however, keeps the microbial production of mannosidostreptomycin at a low level.

The conversion of mannosidostreptomycin into steptomycin was accomplished by enzymatic action of cell-free preparations from a streptomycin-producing strain of *S. griseus* (45), and it is probable that this process will find commercial application. The enzyme, *mannosidostreptomycinase*, is not present in significant quantities in a number of commercial enzyme preparations.

Dihydrostreptomycin

Studies on the elucidation of the structure of streptomycin led to the observation that the compound adds two hydrogen atoms when subjected to conditions of catalytic hydrogenation. Reports from three laboratories appeared about the same time describing the hydrogenation of streptomycin and the properties of the dihydrostreptomycin (46, 47, 48). Dihydrostreptomycin was obtained as the crystalline trihelianthate (46) and crystalline reineckate (48). Although the preparation of analytically pure dihydrostreptomycin trihydrochloride was described, the isolation of crystalline dihydrostreptomycin hydrochloride and crystalline dihydrostreptomycin sulfate has been accomplished only recently (39).

Dihydrostreptomycin is qualitatively very similar to streptomycin with regard to chemotherapeutic and pharmacological effects. The dihydro compound assumed particular importance with the discovery that vestibular dysfunction appears much later and is less severe with prolonged administration of dihydrostreptomycin than with streptomycin under similar conditions.

The reduction of streptomycin occurs at the aldehyde group, and the resultant dihydrostreptomycin is more stable than the precursor. Dihydrostreptomycin does not respond to any of the carbonyl reactions charac

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TABLE 6

Half life of pure streptomycin under different conditions of pH and temperature (20)

pH	HALF LIFE AT			
	7°C	28°C	50°C	95°C
	hours	*hours*	*hours*	*hours*
0.8	1200	110	8	
1.7	Stable	1500	90	
2.7	Stable	Stable	900	
5.5	Stable	Stable	4600	37
7.0	Stable	Stable		
8.6	Stable	1100	50	
9.5	3000	300	28	
11.2		16		

Compounds Related to Streptomycin

Mannosidostreptomycin

Fried and Titus (40), during a study of the alumina chromatography of streptomycin concentrates, obtained in addition to active fractions of high streptomycin content a number of less active, more firmly adsorbed fractions having a microbial potency of 150 to 200 μg/mg. The behavior of these fractions toward a modified Craig countercurrent distribution technique (14) and toward alkali suggested that they contained a new antibiotically active base closely related to streptomycin. The isolation of this new substance, named by the authors *streptomycin B*, was accomplished by treatment of chromatographic fractions with reinecke salt, whereby a crystalline trireineckate was obtained. After several recrystallizations of the latter from warm water, the reineckate was converted into streptomycin B sulfate by reaction with silver sulfate (36). Structural studies by Fried and Stavely (42) established that streptomycin B contains the structural elements of streptomycin joined glycosidically to D-mannose. This latter observation gave rise to the name *mannosidostreptomycin*.

Streptomycin and mannosidostreptomycin differ in their antibiotic microbial spectrum (43). Against *Kl. pneumoniae*, streptomycin is about 3.7 times as active as mannosidostreptomycin, and against *B. subtilis* the ratio

is about 5 to 1. The two compounds form approximately the same quantity of maltol on treatment with alkali. By comparing the maltol color assay (28) with the microbial assay (*B. subtilis*) it is possible to calculate with some degree of accuracy the quantity of mannosidostreptomycin in streptomycin. The quantitative estimation of the two in a mixture can be made by the modified Craig countercurrent distribution technique (36, 14). A colorimetric assay for mannosidostreptomycin in mixtures with streptomycin, which depends upon a color reaction of mannose, was published recently (44).

Streptomycin remains the drug of choice, since little is known about the pharmacology of mannosidostreptomycin. At present the latter is generally separated from streptomycin during the purification steps, but this practice represents an economic loss. Control of the fermentation process, however, keeps the microbial production of mannosidostreptomycin at a low level.

The conversion of mannosidostreptomycin into steptomycin was accomplished by enzymatic action of cell-free preparations from a streptomycin-producing strain of *S. griseus* (45), and it is probable that this process will find commercial application. The enzyme, *mannosidostreptomycinase*, is not present in significant quantities in a number of commercial enzyme preparations.

Dihydrostreptomycin

Studies on the elucidation of the structure of streptomycin led to the observation that the compound adds two hydrogen atoms when subjected to conditions of catalytic hydrogenation. Reports from three laboratories appeared about the same time describing the hydrogenation of streptomycin and the properties of the dihydrostreptomycin (46, 47, 48). Dihydrostreptomycin was obtained as the crystalline trihelianthate (46) and crystalline reineckate (48). Although the preparation of analytically pure dihydrostreptomycin trihydrochloride was described, the isolation of crystalline dihydrostreptomycin hydrochloride and crystalline dihydrostreptomycin sulfate has been accomplished only recently (39).

Dihydrostreptomycin is qualitatively very similar to streptomycin with regard to chemotherapeutic and pharmacological effects. The dihydro compound assumed particular importance with the discovery that vestibular dysfunction appears much later and is less severe with prolonged administration of dihydrostreptomycin than with streptomycin under similar conditions.

The reduction of streptomycin occurs at the aldehyde group, and the resultant dihydrostreptomycin is more stable than the precursor. Dihydrostreptomycin does not respond to any of the carbonyl reactions charac

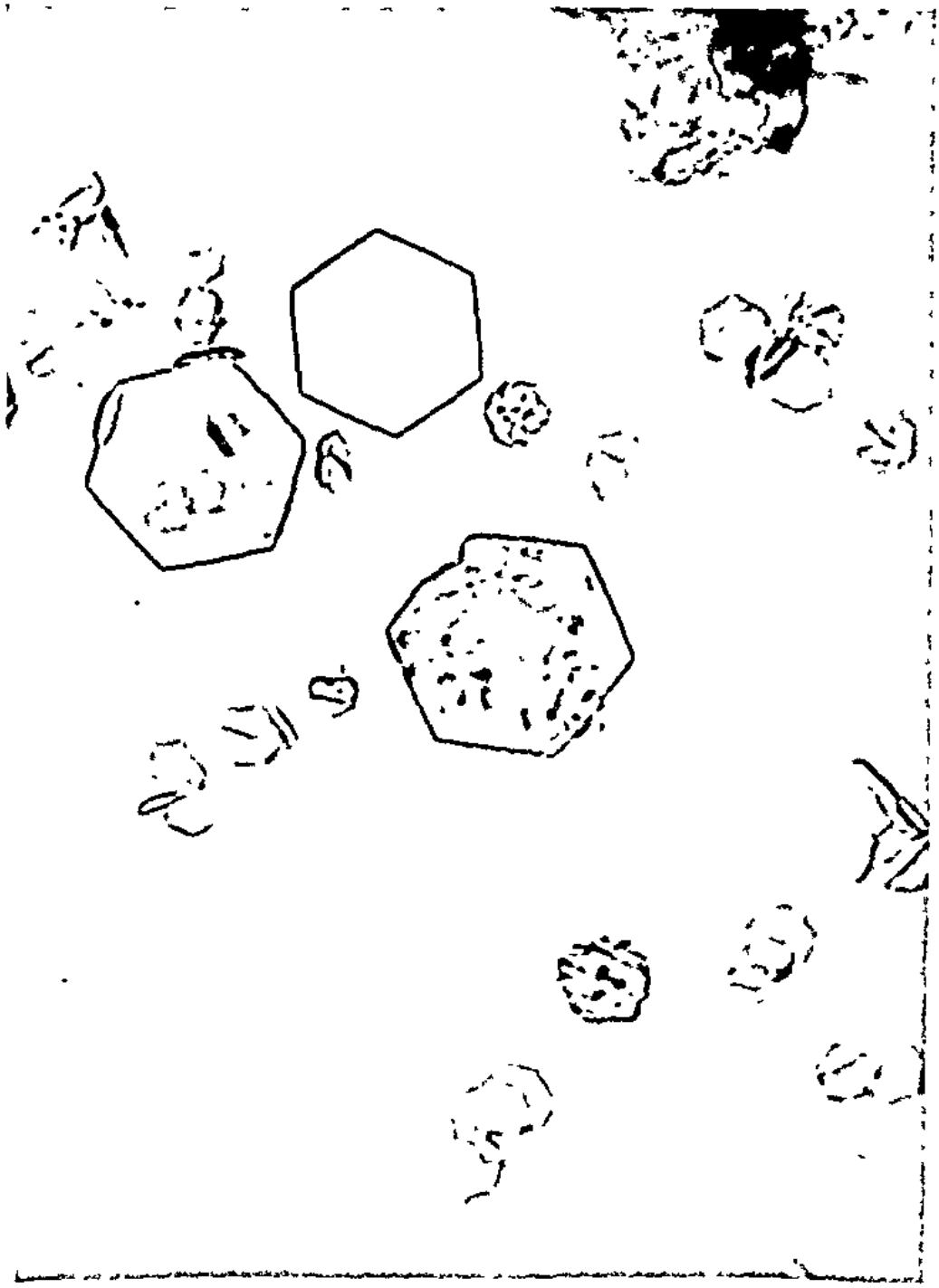


FIG. 11. Crystalline mannosidostreptomycin hydrochloride (Courtesy of E T Stiller (38))

teristic of streptomycin. Unlike the latter, dihydrostreptomycin does not form maltol on alkali treatment, and this difference can be used to measure

the course of hydrogenation and the quantity of streptomycin in samples of dihydrostreptomycin.

The hydrogenation of streptomycin may be carried out in water solutions with platinum oxide or palladium black as a catalyst under hydrogen pressures below 20 pounds/square inch. Both the crystalline calcium chloride complex and pure streptomycin hydrochloride have been hydrogenated successfully; and in the latter instance, essentially pure dihydrostreptomycin hydrochloride is obtained by lyophylizing the filtered reaction

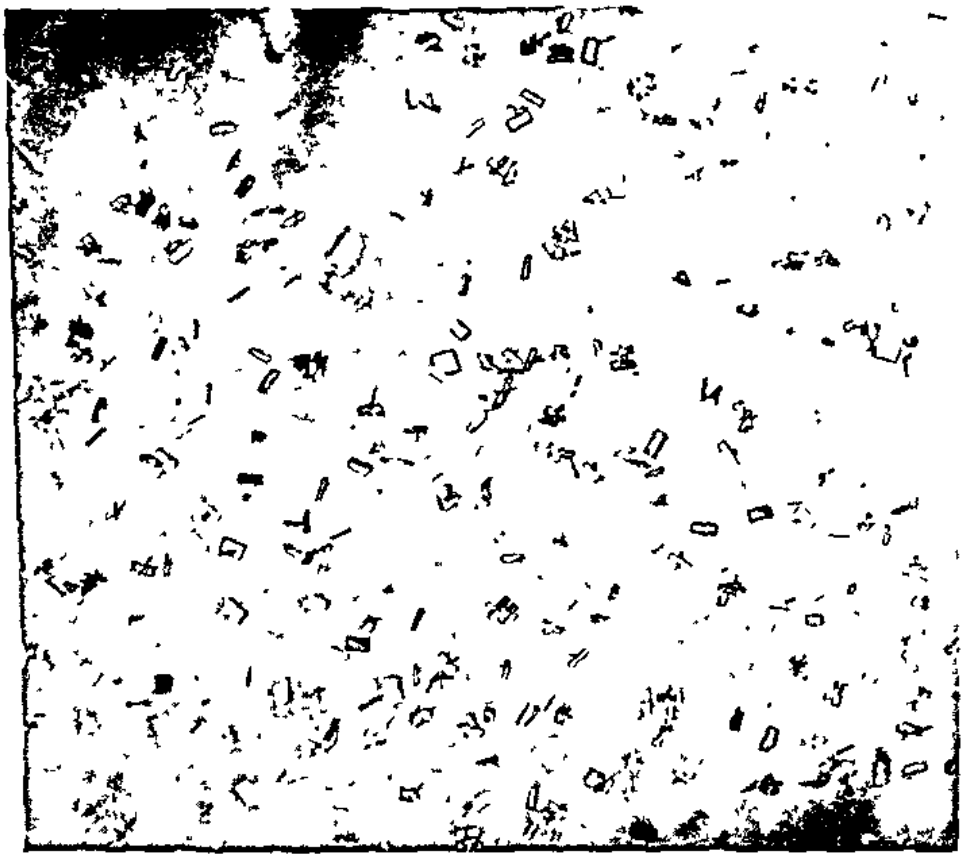
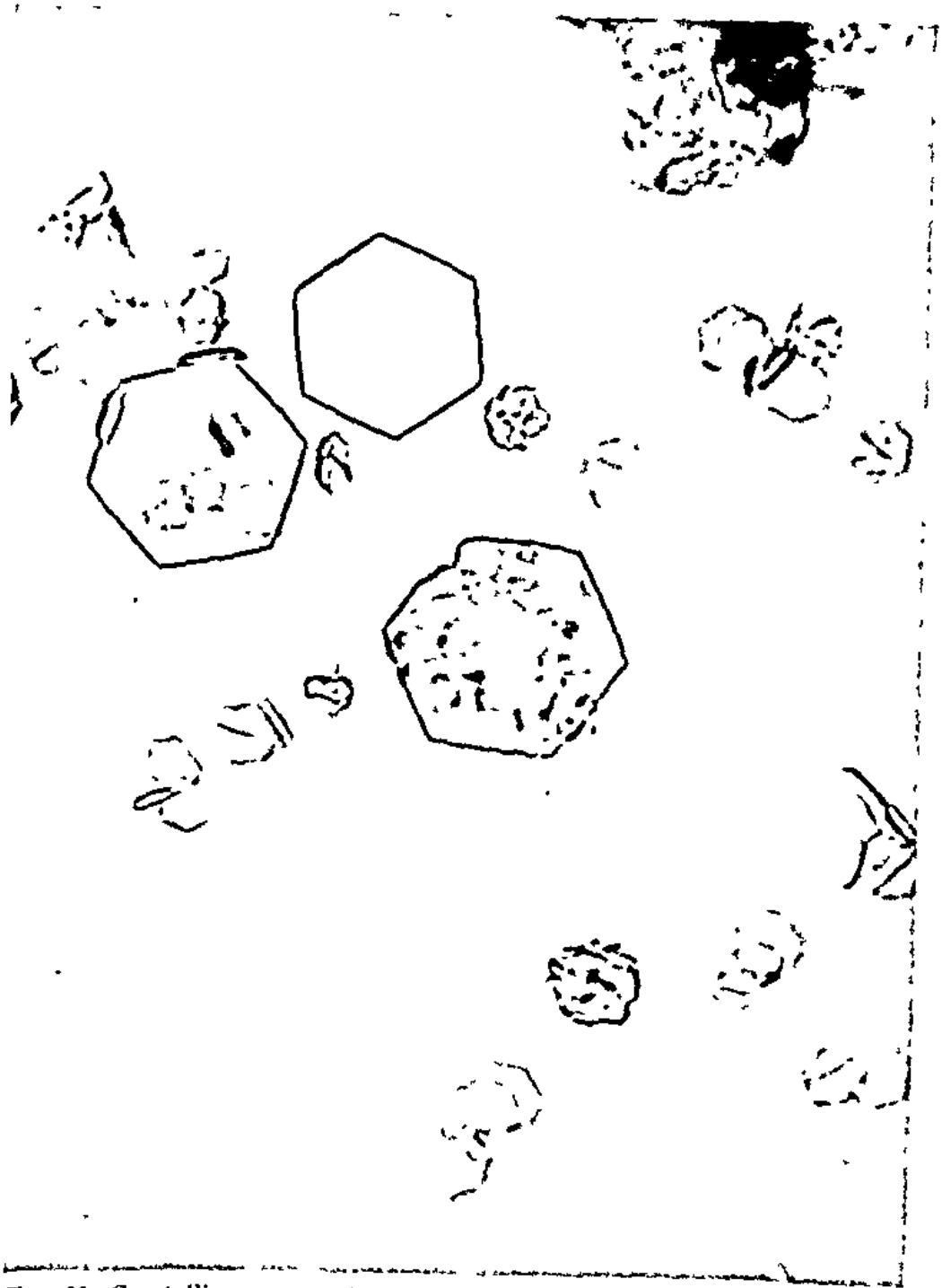


FIG 12 Crystalline dihydrostreptomycin hydrochloride (39)

mixture Since dihydrostreptomycin does not form a crystalline calcium chloride complex (48), the use of streptomycin calcium chloride complex for the preparation of dihydrostreptomycin requires the removal of calcium either before or after the hydrogenation step.

The catalytic hydrogenation of streptomycin has been adapted to large-scale production, and today dihydrostreptomycin is readily available as the sulfate and as the hydrochloride.

Mannosidostreptomycin undergoes catalytic hydrogenation under the same conditions developed for streptomycin (36). To date, no pharmacological or clinical data for dihydromannosidostreptomycin have appeared.



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Certification of Streptomycin and Dihydrostreptomycin

In the interest of the public welfare, introduction of a new drug today requires approval of the Food and Drug Administration. To obtain this approval the producer must present to this governmental agency the chemical, pharmacological, and clinical data that establish the effectiveness and safety of the new medicinal. Approval must also be obtained for every new pharmaceutical preparation containing the drug.

The Food and Drug Administration is also responsible for establishing the specifications for a new drug and for preventing the sale of drugs that do not conform to these specifications. This agency also has the right to set up a system of certification, which requires that samples of all lots of a given drug must be examined and found satisfactory by the Food and Drug Administration before the particular lot can be sold.

The provisions of Section 507 of the Food, Drug and Cosmetic Act (52 Stat. 1040, 1055, as amended by 59 Stat. 463 and 61 Stat. 11; 21 U.S.C. Supp. 357) require that each batch of penicillin or streptomycin or of a derivative be certified by the Federal Security Administration (49). To be certified, each batch must have the characteristics of quality and purity established by the regulations that ensure safety and efficacy. At present, these certification regulations apply to all samples of penicillin, streptomycin, and dihydrostreptomycin offered for sale, even if these are pure.

Streptomycin specifications

Certification for sale of a lot of streptomycin is obtained by submitting to the Food and Drug Administration a complete duplicate analysis of the lot. These analyses cover the specifications (50) listed below. From each lot the producer submits to the Food and Drug Administration as samples five vials for every 25,000 vials of drug and one additional vial for every 5,000 vials up to a maximum of twelve vials. These samples are tested by the Food and Drug Administration, and if the samples meet the established specifications, the lot of streptomycin is certified for sale. The lots must pass the following specifications:

Potency. The potency of the material must be greater than 300 μg/mg. The potency of the streptomycin is measured by its antibiotic activity against a standard strain of *B. subtilis* or *Kl. pneumoniae* with either the cup-diffusion method or the turbidimetric method of assay.

Sterility. The lot must be sterile. The sterility test involves the incubation at 37°C for 4 days of a medium containing streptomycin and a substance known to inactivate streptomycin (such as thioglycollic acid or hydroxylamine). One tube in the test is inoculated with *Kl. pneumoniae.* The inoculated tube should produce growth, whereas the other tubes should be devoid of growth.

Finishing Operations

After extensive chemical processing of a streptomycin or dihydrostreptomycin salt to ensure purity according to chemical and pharmacological standards, there still remains the complex steps of a finishing operation for the production of a clinically acceptable form of the drug. Streptomycin and dihydrostreptomycin salts must be made available to the medical profession as sterile powders contained in a sterilized vial, which can be used conveniently for the preparation of solutions suitable for parenteral use. To ensure complete sterility, the purified products are processed through additional steps for removal of spores and bacteria. This is done by passing a concentrated solution of the streptomycin or dihydrostreptomycin salt through bacterial filters; and sterile techniques are employed throughout this and subsequent operations. The sterilized solution is concentrated to dryness in trays or in sterilized vials. In the latter instance, a measured amount of solution is placed in each ampule before concentration. The removal of water from the solution is carried out by the lyophylization procedure, which was developed previously for sensitive biologicals and applied successfully to the processing of penicillin. Solutions of streptomycin or dihydrostreptomycin in this method are frozen and chilled to −40°C, and the frozen concentrate is placed in a high vacuum dryer at less than 100 μ of pressure. Sublimation of the ice occurs, and the water vapors from the dryer pass to a condenser maintained at −80°C. The vapor collects on the condenser walls as ice and is scraped off regularly as required.

When the lyophylization is carried out on trays, the product is removed from the trays, milled, and blended. Again these operations are all carried out under aseptic conditions. The product is tested according to the rigid specifications of the Food and Drug Administration; and if the lot conforms to these specifications, it is subdivided into ampules under sterile conditions in low humidity sterile cabinets Since streptomycin preparations are sold and used on the basis of their free streptomycin content, vials containing the equivalent of 1 gm of free base actually contain more than this quantity of solid. One gram of streptomycin base is contained in 1 19 gm of pure streptomycin trihydrochloride, in 1.25 gm of pure streptomycin sulfate, and in 1.28 gm of pure streptomycin calcium chloride complex. With less pure samples of these three salts, the weights equivalent to 1 0 gm of streptomycin are greater than the values just noted. The label on the vial always indicates the weight of streptomycin base contained in the vial but does not generally state the actual weight of the contents. Unless one weighs the contents of the vial or assays a sample, it is not possible to ascertain the purity of a given sample, except for known averages.

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Toxicity. A quantitative acute toxicity test based on Ott's (52) procedure is employed. This procedure consists in injecting each of five mice, weighing 18 to 25 gm, with a test dose of 0.5 ml of a solution containing 2 mg of streptomycin base per cubic centimeter of solution. The injection should be made over a period of not more than 5 seconds. If no animal dies within 48 hours, the sample is considered satisfactory. If one or more animals die within 48 hours, the test is repeated with five additional mice; if all animals survive the repeat test, the sample is acceptable.

Histamine-like substance. The product is tested for blood-depressor factors by intravenous injection into an anesthetized cat. A dose of 3 mg of base should produce a drop in blood pressure of not more than that produced by 0.10 μg of histamine per kilogram of body weight.

Moisture. The lot should contain not more than 3.0 per cent moisture as determined by drying a sample in a vacuum oven at 60°C., at a pressure of 5 mm. of mercury or less for 3 hours.

pH. An aqueous solution of 0.2 gm base per milliliter should have a pH between 4.5 and 7.0.

Solubility. Solutions prepared by adding 0.2 gm of streptomycin base to 1 ml of water, to 5 per cent dextrose solution, or to physiological saline solution should be substantially free from turbidity or undissolved material.

Pyrogens. The lot must not give a positive pyrogen reaction when 10 mg of streptomycin base per milliliter is injected into rabbits. A positive reaction is indicated by a temperature rise of 0.6°C or more. A more quantitative method of determining pyrogens will be published shortly by W. H. Ott (41).

Dihydrostreptomycin specifications

Certification before sale is required for dihydrostreptomycin (33). Each lot of the drug must conform to the specifications established for streptomycin except that:

The potency[3] must not be less than 600 μg/mg.

The content of streptomycin sulfate or streptomycin hydrochloride must not exceed 3 per cent when calculated as streptomycin base. The streptomycin content is established by the ferric-maltol colorimetric assay.

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[3] Pure dihydrostreptomycin sulfate has a potency of 800 μg/mg., and pure dihydrostreptomycin hydrochloride has a potency of 840 μg/mg.

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CHAPTER 5

THE CHEMISTRY OF STREPTOMYCIN

It is the purpose of this review to describe some of the salient and primary features of the chemical research on streptomycin so that this information can be readily perused for a general knowledge of the subject. It is neither intended nor possible to review all of the chemical literature on streptomycin, or to present an exhaustive and detailed review of the organic chemistry in this chapter.

DISCOVERY

A new antibiotic, designated *streptomycin*, was recognized by Schatz, Bugie, and Waksman (1) in 1944 in concentrates from the culture media of two strains of an actinomyces which is related to an organism described today as *S. griseus*. This antibiotic was immediately of great interest, because it exhibited selective activity against gram-negative bacteria. Streptomycin resembled streptothricin (2) in general chemical properties and antibacterial behavior, but streptomycin was later found to be much more therapeutically promising, and consequently was subjected to extensive chemical, biological, and clinical research. However, the elucidation of the structure of streptothricin would be of interest for a better understanding of the relationship between the chemical structure and the antibiotic activity of the two compounds.

ISOLATION

Several laboratories initiated research on the isolation of streptomycin as a homogeneous substance, and within a year after the discovery of the antibiotic, it was isolated in the form of pure crystalline salts.

Fried and Wintersteiner (3) succeeded in obtaining a crystalline reineckate which permitted analytical determinations on a pure salt. Kuehl, Peck, Walti, and Folkers (4) were also successful in obtaining a crystalline salt of streptomycin when they treated concentrates of streptomycin hydrochloride with the sodium salt of helianthine (methyl orange) and found that streptomycin helianthate crystallized from the solution. The pure strepto-

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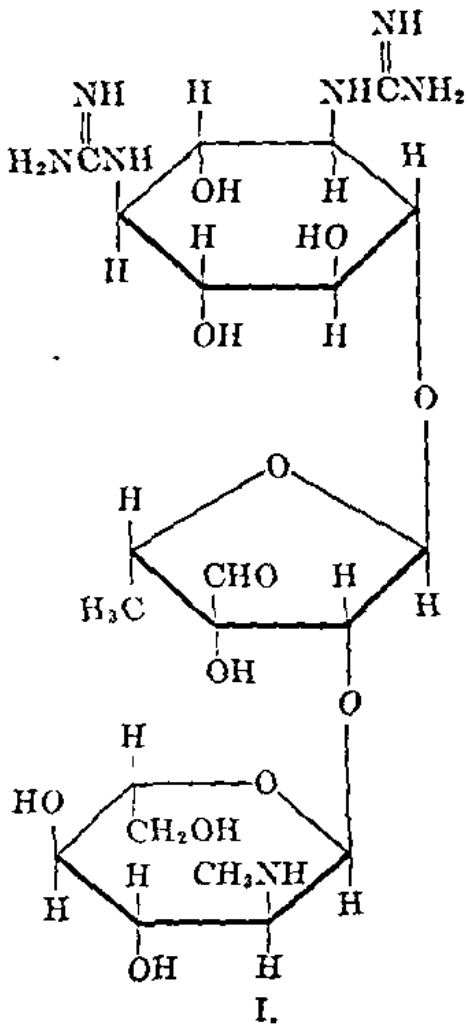
mycin helianthate was readily and satisfactorily converted into the hydrochloride or sulfate for chemical, biological, or therapeutic purposes.

Shortly after the characterization of the crystalline streptomycin helianthate, it was found by Peck, Brink, Kuehl, Flynn, Walti, and Folkers (5) that streptomycin hydrochloride or helianthate and calcium chloride formed a crystalline double salt containing two moles of streptomycin trihydrochloride and one mole of calcium chloride. In addition to providing another pure form of streptomycin for the chemical investigations, the double salt is useful therapeutically, and is marketed commercially for clinical use.

In 1948, Heuser, Dolliver, and Stiller (6) described some properties of crystalline streptomycin trihydrochloride dihydrate.

Structure of Streptomycin

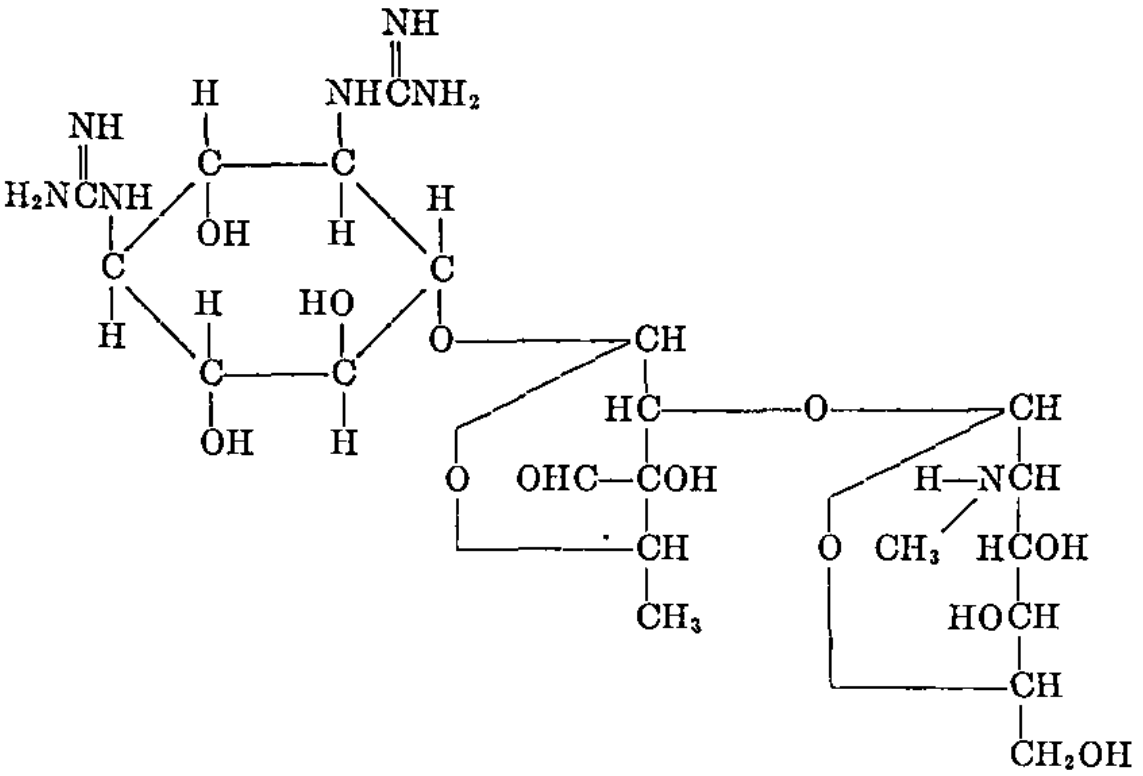
The combined chemical and stereochemical evidence from several laboratories now permits one to write formula I for the structure of streptomycin. Although this structure is complete, including the configuration of all



I.

the asymmetric centers, confirmatory evidence for certain details of structure is desirable. Formula I is a Haworth perspective formula, which has certain advantages for visualizing stereochemical relationships. The structure of streptomycin may also be represented by formula II in the more common Fischer convention.

Streptomycin is derived from three substances, streptidine, streptose, and N-methyl-L-glucosamine. These have the structures III, IV, and V, respectively. A hypothetical reaction of these three substances, which perhaps takes place at least in part biogenetically, with the elimination of two molecules of water yields streptomycin.



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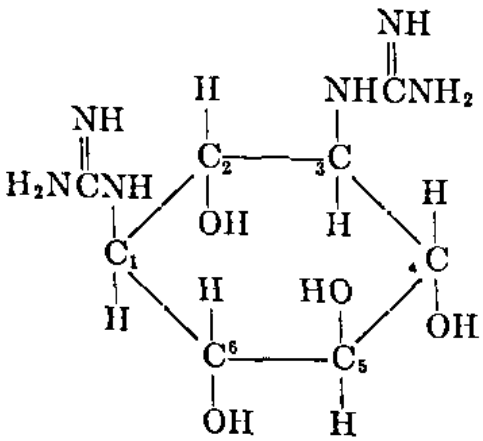

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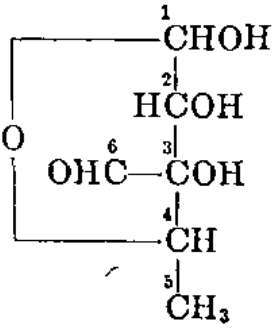
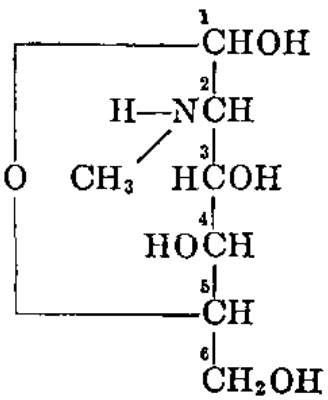
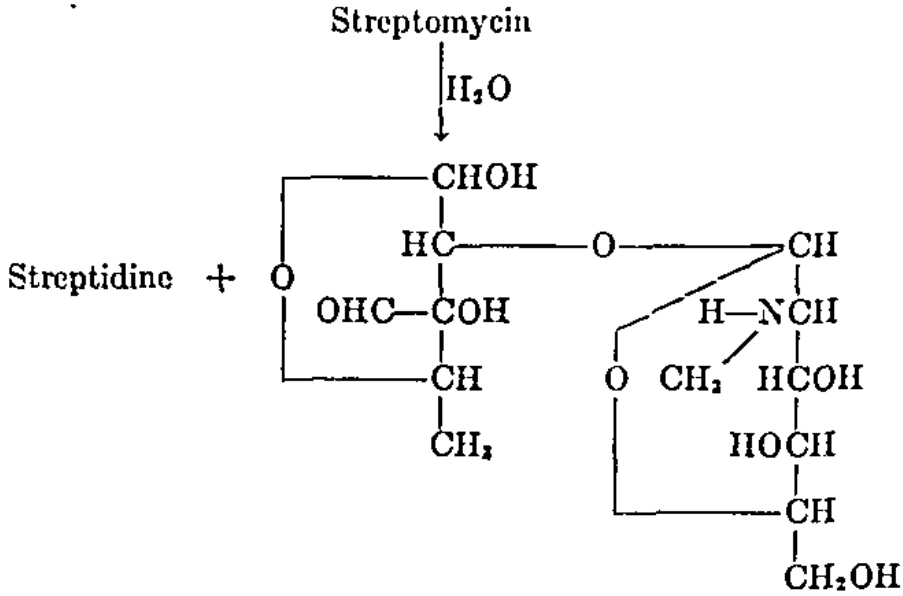


IV.



V.

The glycosidic linkage between the streptidine and streptose moieties of streptomycin is much weaker than that between the streptose and N-methyl-L-glucosamine portions. Consequently, streptomycin may be cleaved by a variety of reactions to yield streptidine plus the streptose and N-methyl-L-glucosamine moieties still joined glycosidically as a disaccharide. This disaccharide was designated *streptobiosamine* (VI).



VI.

In the elucidation of the structure of a new complex organic compound, those degradation reactions which are frequently useful are acid and alkaline hydrolysis, oxidation, and reduction. Cleavage of a molecule by these methods results in the formation of smaller fragments which may be identifiable as such, or which may in turn be further degraded by one or more reactions to yield recognizable fragments From the identities of the various simpler products obtained, and the natures of the reactions by which they were prepared, the constitution of the original molecule is deduced. All of these modes of attack and others were applied to streptomycin. Acid hydrolysis and the related methanolysis and mercaptolysis reactions were very productive Alkaline hydrolysis resulted in a molecular rearrangement of the streptose portion of the antibiotic. Mild oxidation and reduction reactions did not lead directly to cleavage of the molecule.

Molecular formula and properties

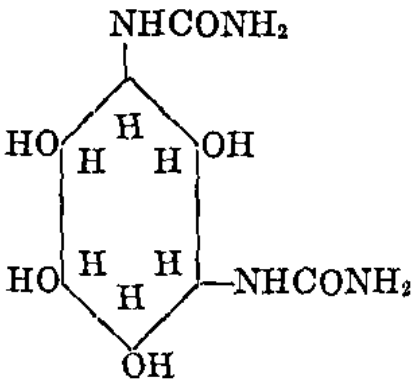
Elementary analyses and potentiometric titrations on a number of crystalline salts of streptomycin, together with a molecular weight determination on the amorphous hydrochloride, gave results in agreement with the

formula $C_{21}H_{37-39}N_7O_{12}\cdot 3HX$ for streptomycin salts as reported by Brink, Kuehl, and Folkers (5, 7). This molecular formula was confirmed by Fried and Wintersteiner (8) from analytical data on streptomycin reineckate, and by Hooper, Klemm, Polglase and Wolfrom (9) from their data on the streptomycin trihydrochloride calcium chloride double salt. It was not until the compositions of streptidine and of some streptobiosamine derivatives could be interpreted together that the formula $C_{21}H_{39}N_7O_{12}$ for streptomycin was definitely established by Kuehl, Flynn, Brink, and Folkers (10).

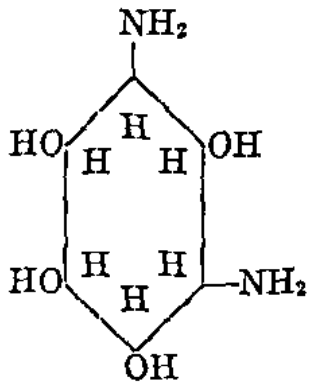
In addition to the three basic groups shown by titration, streptomycin contains a reactive carbonyl group. Its presence was shown by the inactivation of the antibiotic by carbonyl group reagents, and by the preparation of streptomycin oxime and semicarbazone (7).

Streptidine

Aqueous acid hydrolysis of streptomycin yielded the diacidic base streptidine, $C_8H_{18}N_6O_4$ (formula III) according to Peck, Graber, Walti, Peel, Hoffhine, and Folkers (11). Stepwise alkaline degradation of streptidine gave first a urea derivative, strepturea (VII), and then a diamine, streptamine (VIII). Acetylation of streptamine yielded a hexaacetyl derivative. Permanganate oxidation of streptidine afforded 1.3 moles of guanidine.



VII.



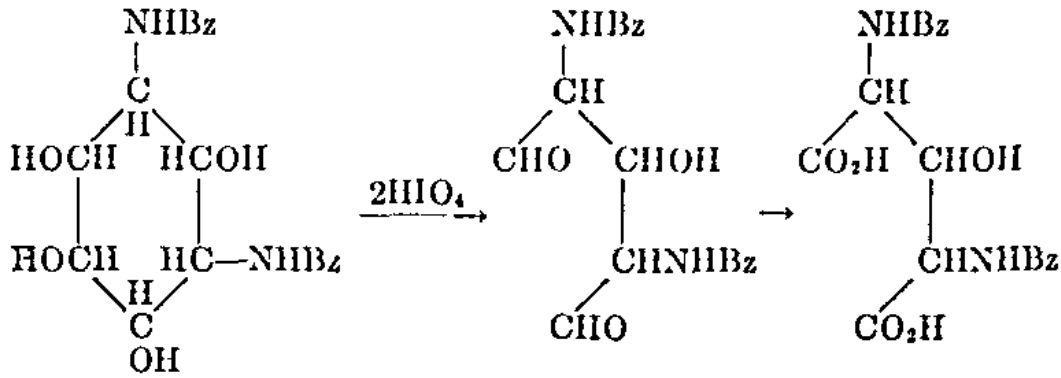
VIII.

Thus, all of the nitrogen and oxygen atoms of streptidine were accounted for by two guanido groups and four hydroxyl groups. The absence of unsaturation suggested a cyclic skeleton, presumably six-membered, bearing four hydroxyl groups, and two guanido groups in the 1,2-, the 1,3-, or the 1,4-positions.

Proof that the 1,3-diguanido structure (III) is correct was obtained by Carter, Clark, Dickmann, Loo, Skell, and Strong (12) and by Peck, Hoffhine, Peel, Graber, Holly, Mozingo, and Folkers (13).

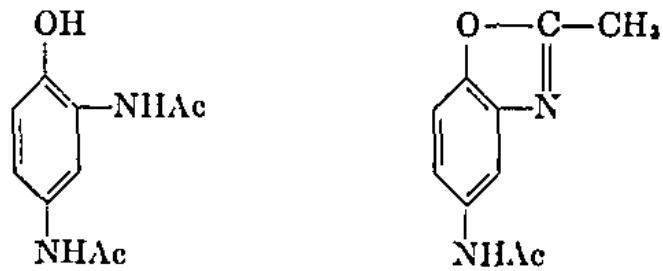
Periodic acid oxidation of N,N′-dibenzoylstreptamine (IX) gave a crys-

talline five-carbon dialdehyde (X), which in turn yielded on bromine oxidation a dibenzamidohydroxyglutaric acid (XI). Only structure IX for N,N′-dibenzoylstreptamine could give these results, showing that formula III is correct (12).



IX. X. XI.

Pyrolysis of hexaacetylstreptamine gave high yields of 2,4-diacetamidophenol (XII) and 5-acetamido-2-methylbenzoxazole (XIII), proving also that streptidine has structure III (13).



XII. XIII.

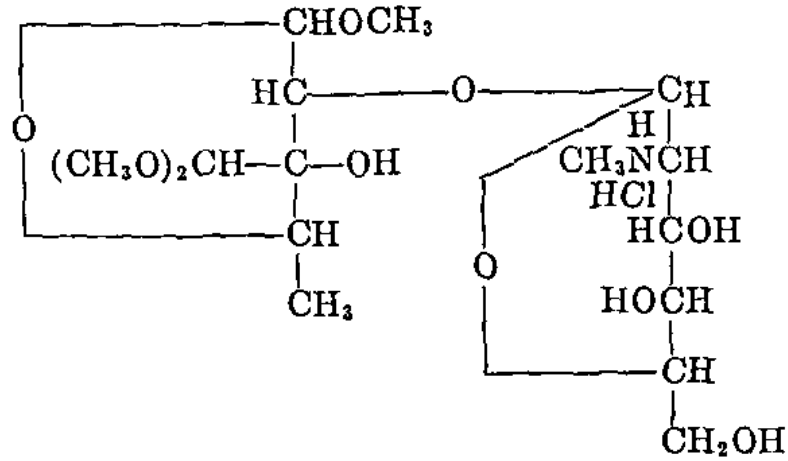
Streptidine is optically inactive, and it was concluded that it must be a meso form

The structure of streptidine as a 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane was confirmed, and its stereochemical configuration elucidated by a synthesis of streptamine from D-glucosamine by Wolfrom and Olin (14). They concluded that streptidine possesses the all-*trans* configuration, as indicated in formula III The configuration about C_2 was deduced by analogy, and supporting evidence is desirable

N-methyl-L-glucosamine

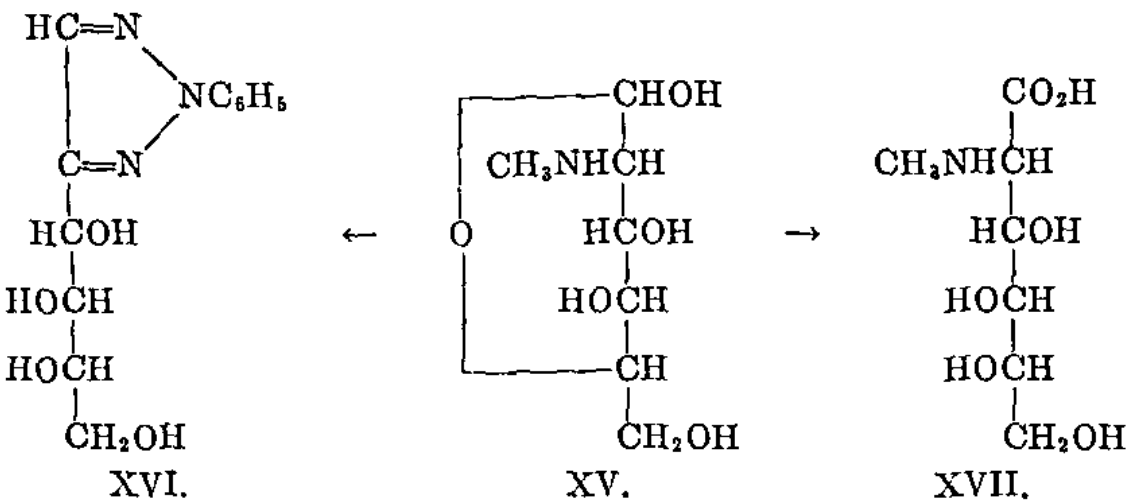
Hydrolysis in boiling concentrated hydrochloric acid of methyl streptobiosaminide dimethyl acetal (XIV), a derivative of streptobiosamine obtained (7, 15) by methanolysis of streptomycin, led to decomposition of the streptose moiety of the disaccharide and the isolation, after acetylation, of

she pentaacetyl derivative of a methylaminohexose. This compound was readily hydrolyzed to the free sugar, (XV). The new sugar gave a phenyl-



XIV.

osazone which was converted to a phenylosotriazole (XVI). This derivative had the same melting point as that of the known D-glucose phenylsotriazole, and the specific rotation was equal in magnitude but opposite in



XVI. XV. XVII.

sign. Mercuric oxide oxidation of the hexosamine gave a nitrogen-containing acid, XVII, having the same melting point as the reported N-methyl-D-glucosamic acid and an equal but opposite specific rotation It was thus concluded by Kuehl, Flynn, Holly, Mozingo, and Folkers (16) that the hexosamine from streptomycin was N-methyl-L-glucosamine (XV).

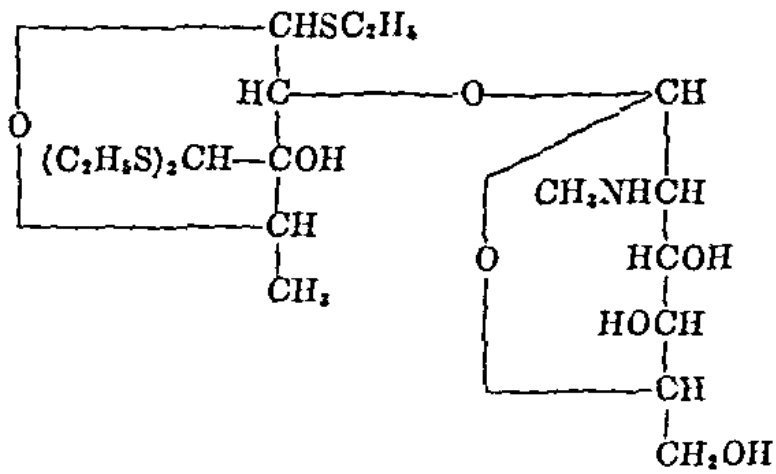
The structure of N-methyl-L-glucosamine was confirmed (16) by its synthesis from L-arabinose, and by the conversion of D-glucosamine to N-methyl-D-glucosamine hydrochloride, which showed the same melting point as the hexosamine from streptomycin and which had specific rotation of equal magnitude but opposite sign.

Streptose

Because of its instability, the streptose portion of streptomycin offered the greatest experimental difficulties, and its structure was the last to be elucidated. Streptose itself was not isolated, although several oxidized or reduced derivatives have been described.

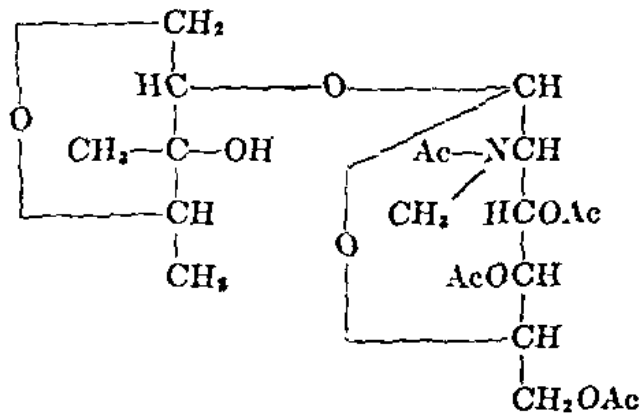
However, even before the isolation of any streptose derivative, considerable knowledge regarding the nature of this fragment was available, and the formula of streptose was calculated to be $C_6H_{10}O_5$, based on the known formulas of streptomycin and of streptidine and the hexosamine.

The determination of the structure of streptose resulted from the isolation of crystalline derivatives obtained by reduction and oxidation. For both degradations, the starting material was the triethylmercapto derivative, XVIII, of streptobiosamine.

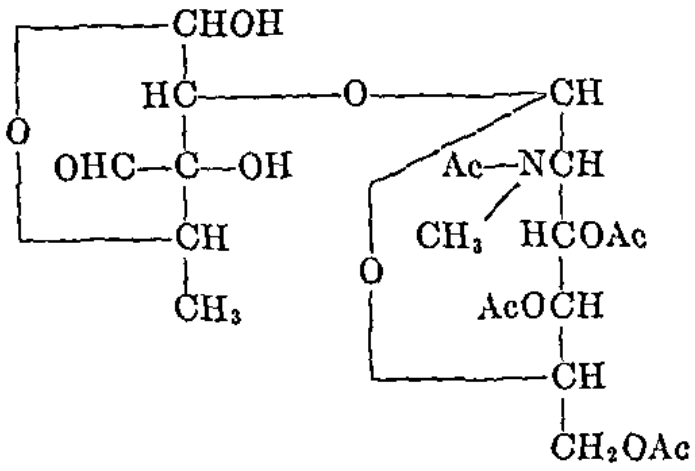


XVIII.

Hydrogenolysis of the acetylated trimercapto derivative with Raney nickel catalyst gave tetraacetylbisdesoxystreptobiosamine (XIX). Hydrolysis of the ethylmercapto groups by aqueous mercuric chloride solution gave tetraacetylstreptobiosamine (XX). Hydrolysis of tetraacetylbisde-

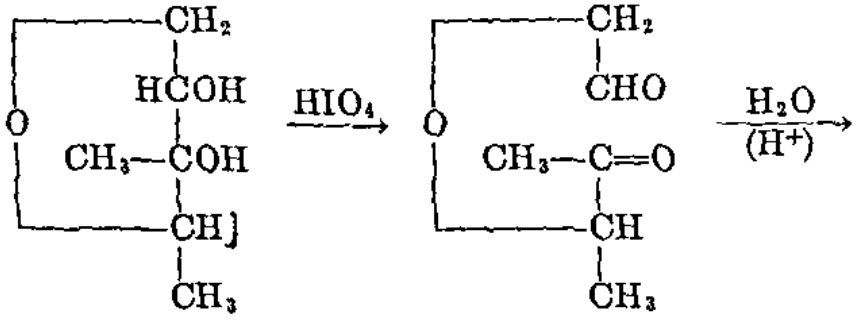


XIX.

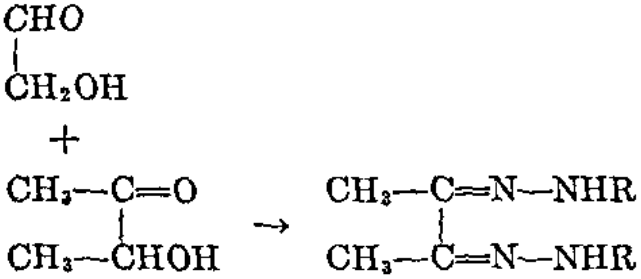


XX.

soxystreptobiosamine by sulfuric acid afforded N-methyl-L-glucosamine and a new compound, $C_6H_{12}O_3$. This compound was designated *bisdesoxystreptose* and was shown by Brink, Kuehl, Flynn, and Folkers (17, 18) to have structure XXI. Bisdesoxystreptose contains two C-methyl groups and two hydroxyl groups. It was found to react with one mole of periodic



XXI. XXII.

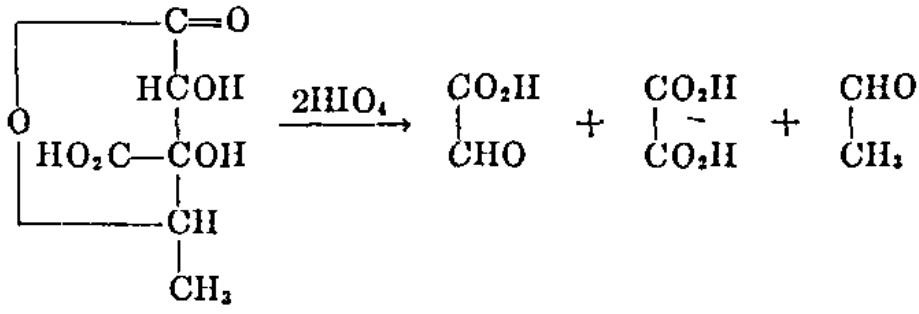


XXIII.

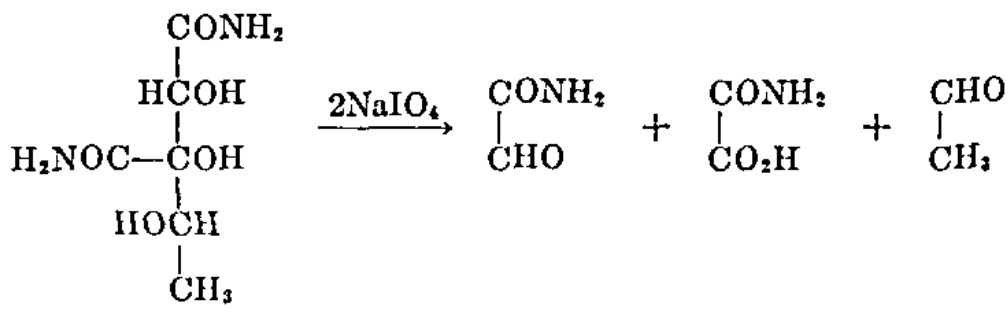
acid to yield a dicarbonyl compound $C_6H_{10}O_3$, XXII. This product was readily hydrolyzed by mild acid treatment; the acetoin, which was formed, was characterized by the preparation of several osazones of biacetyl, XXIII. Bisdesoxystreptose formed an acidic complex with boric acid, indicating a *cis* configuration of the two hydroxyl groups.

When tetraacetylstreptobiosamine (XX) was oxidized with bromine water, and the product hydrolyzed with acid, a new lactone, $C_6H_8O_6$, was

produced. This new compound, which was named *streptosonic acid monolactone,* was degraded further and found to have structure XXIV by Kuehl, Flynn, Brink, and Folkers (19). The monolactone was readily converted to a diamide, XXV. The structure of streptosonic acid monolactone and that of the diamide were revealed by a study of their reactions with periodate. Thus, the monolactone reacted with two moles of periodic acid, and glyoxylic and oxalic acids were isolated and identified. Likewise, the diamide upon periodate oxidation consumed two moles of the reagent and yielded acetaldehyde but no volatile acid.



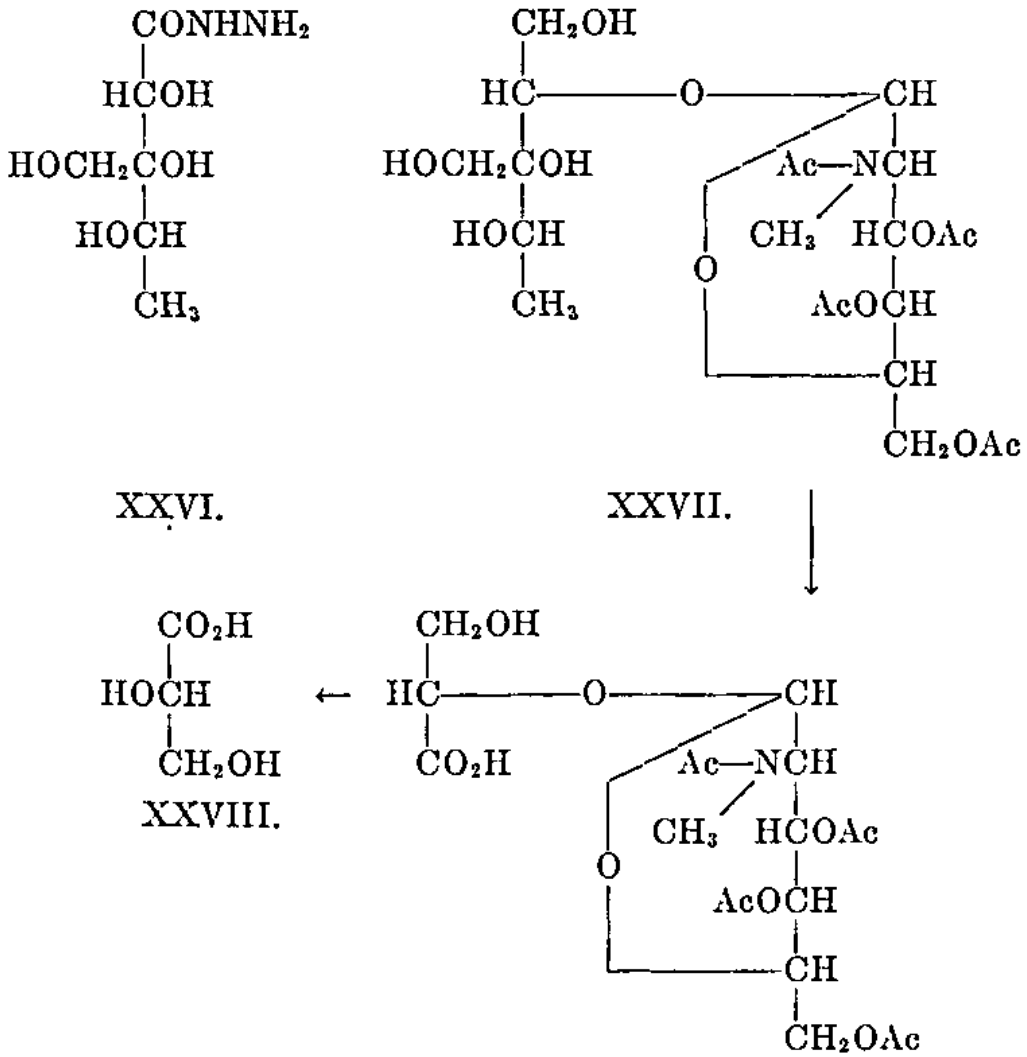
XXIV.



XXV.

From the known structures of bisdesoxystreptose and streptosonic acid, it was apparent that streptose has the constitution shown by formula IV.

Knowledge of the stereochemistry of streptose was forthcoming from several laboratories. Fried, Walz, and Wintersteiner (20) isolated the phenylosazone of 4-desoxy-L-erythrose from streptobiosamine, showing that carbon atom four of streptose possessed the L-configuration. Kuehl, Bishop, Flynn, and Folkers (21) prepared the hydrazide of dihydrostreptosonic acid (XXVI). This compound is dextrorotatory and, in consideration of Hudson's rules of rotation, it was deduced that in streptose the hydroxyl group on carbon atom two is on the right (Fischer formula). Since it had previously been shown that the C_2- and C_3-hydroxyl groups are *cis* (17, 18), the hydroxyl group on carbon atom three is also written to the right. The configuration of the groups about carbon atom two of streptose was confirmed by Wolfrom and DeWalt (22). These workers prepared N-acetyltetrahy-



XXVI. XXVII.

XXVIII.

drostreptobiosamine (XXVII), which was degraded to L-glyceric acid (XXVIII), thus confirming the stereochemical configuration at carbon atom two.

Streptobiosamine

The facile cleavage of streptomycin by acid to yield streptidine and derivatives of streptobiosamine has been mentioned. Methanolysis of streptomycin by Brink, Kuehl, and Folkers (7, 15) gave methyl streptobiosaminide dimethyl acetal hydrochloride (XIV), which upon acetylation furnished the first crystalline derivative of the disaccharide. Cleavage of streptomycin with ethyl mercaptan and hydrogen chloride by Kuehl, Flynn, Brink, and Folkers (23) afforded the analogous ethyl thiostreptobiosaminide diethyl mercaptal (XVIII).

Evidence that streptobiosamine is made up of the two monosaccharides N-methyl-L-glucosamine and streptose has been presented. It was early demonstrated that N-methyl-L-glucosamine was attached to streptose

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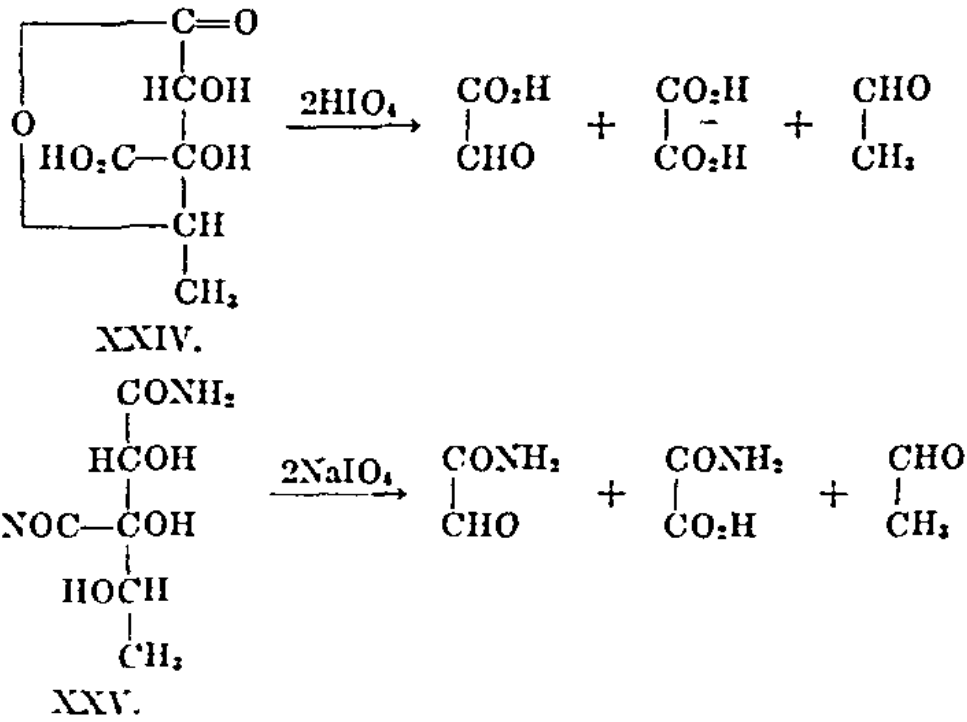

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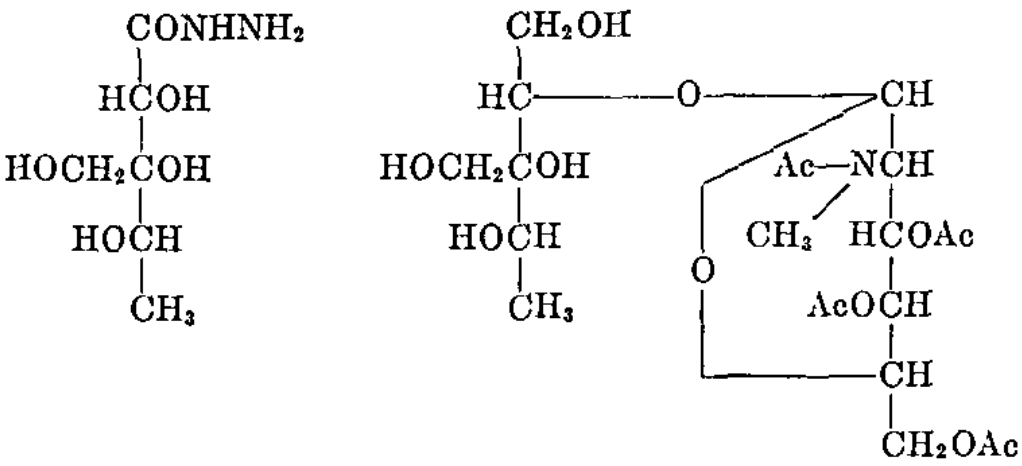
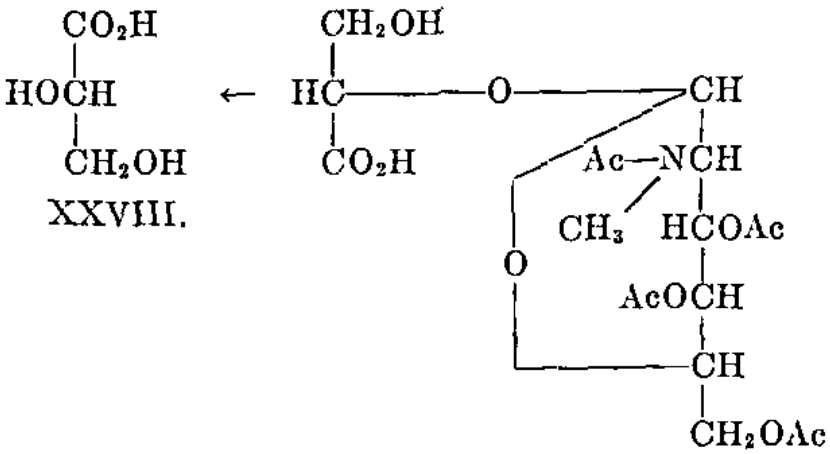


XXVI. XXVII.



XXVIII.

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Evidence that streptobiosamine is made up of the two monosaccharides N-methyl-L-glucosamine and streptose has been presented. It was early demonstrated that N-methyl-L-glucosamine was attached to streptose

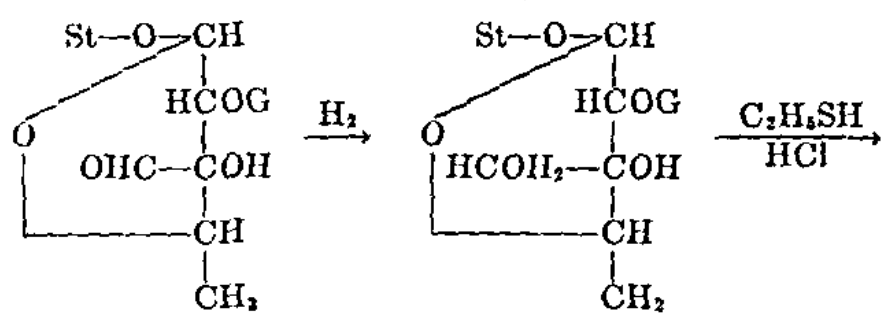
through carbon atom one of the hexosamine, since the N-acetyl derivative of bisdesoxystreptobiosamine did not reduce Fehling's solution (23). A study of tetraacetylbisdesoxystreptobiosamine (XIX) by Brink, Kuehl, Flynn, and Folkers (17, 18) showed that this compound contained a free tertiary hydroxyl group, and hence that N-methyl-L-glucosamine must be attached to carbon atom two of streptose.

The results of periodate oxidation of the N-acetyl derivative of bisdesoxystreptobiosamine (17, 18) suggested that in streptobiosamine, the N-methyl-L-glucosamine moiety had the pyranose ring structure.

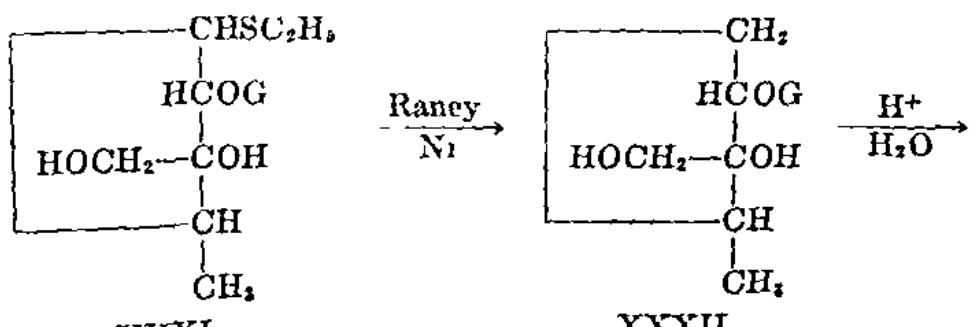
Lemieux, DeWalt, and Wolfrom (21), by the application of Hudson's rules of isorotation to selected derivatives and degradation products of streptomycin, and by the use of certain assumptions, concluded that both of the streptobiosamine glycosidic linkages in streptomycin were of the α-configuration.

Linkage of streptidine to streptobiosamine

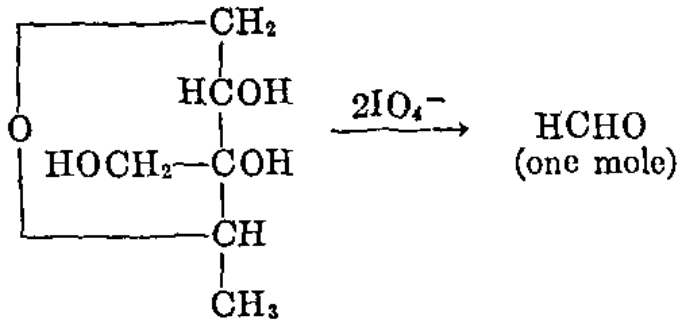
It was evident that streptidine must be glycosidically attached to the disaccharide through either carbon atom one or six of streptose. The proof that streptidine is linked to streptobiosamine through carbon atom one of streptose came finally from a series of degradations, starting with dihydrostreptomycin, which showed that C_6 of streptose belongs to the streptomycin formyl group (18, 23). The key compounds in this degradative series are represented in formulas XXIX to XXXIV (St = streptidine moiety, G = N-methyl-L-glucosamine moiety).



XXIX. XXX.



XXXI. XXXII.



XXXIII. XXXIV.

The results of the reaction of dihydrodesoxystreptose (XXXIII) with periodate were consistent with the previously derived conclusion that the C_1-aldehyde group of streptose was the one involved in the linkage to streptidine.

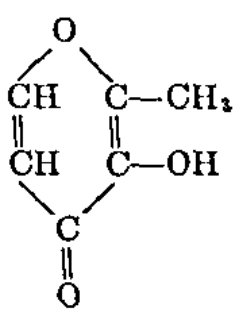
Linkage of streptobiosamine to streptidine

Completely benzoylated streptomycin was cleaved hydrolytically to crystalline heptabenzoylstreptidine (XXXV). From this product were prepared in turn the mesyl derivative, XXXVI, the iodo derivative, XXXVII, and heptabenzoyldesoxystreptidine, XXXVIII. The last compound was converted to N,N′-dibenzoyldesoxystreptamine, XXXIX. The N,N′-dibenzoyldesoxystreptamine reacted with one mole of periodate to form α,γ-dibenzamido-β-hydroxyadipaldehyde, XL. These data, advanced by Kuehl, Peck, Hoffhine, Peel, and Folkers (25, 26, 27) proved that streptobiosamine is attached at position four of streptidine in streptomycin.

This conclusion has been confirmed by Wolfrom and Polglase (28), who studied the periodate consumption of decaacetyldideguanyldihydrostreptomycin, prepared from dihydrostreptomycin by barium hydroxide hydrolysis and acetylation.

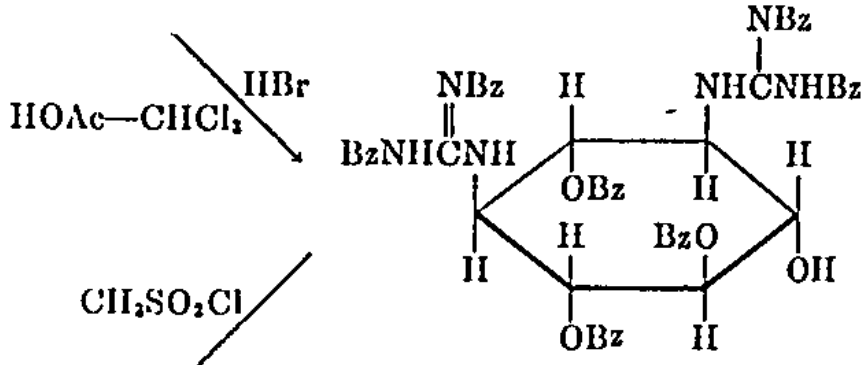
The maltol rearrangement

Schenck and Spielman (29) observed that when streptomycin was treated with aqueous alkali under relatively mild conditions, the γ-pyrone maltol, XLI, was formed. It was evident that maltol originated from the streptose

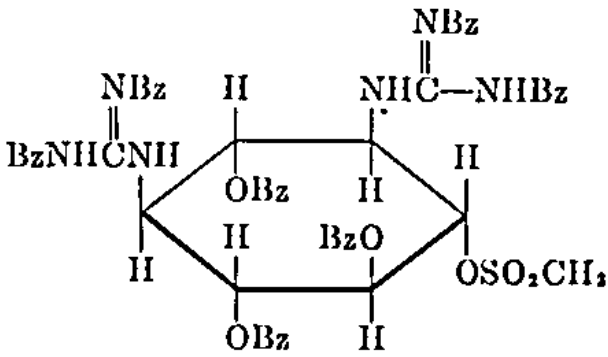


XLI.

Undecabenzoyl-streptomycin

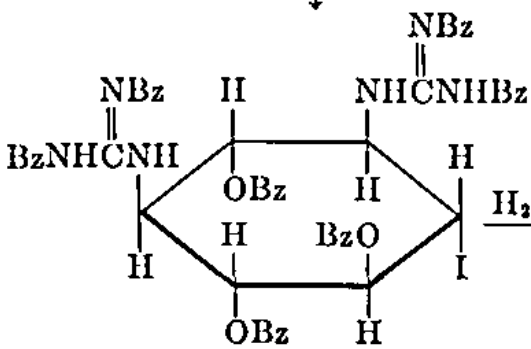


XXXV.
(Bz = benzoyl)



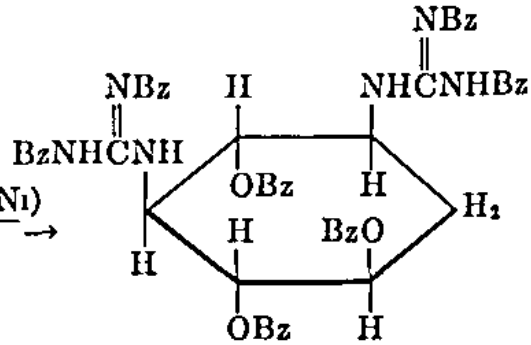
XXXVI.

NaI



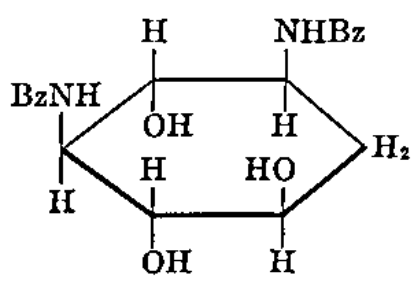
XXXVII.

H_2(Ni)



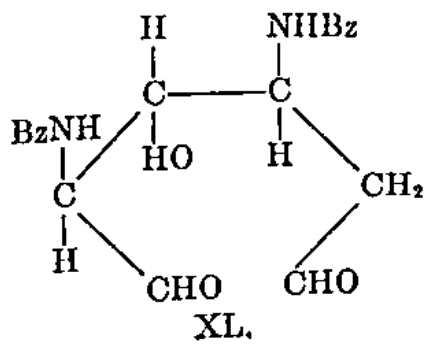
XXXVIII.

(a) hydrolysis
(b) benzoylation
(c) partial debenzoylation



XXXIX.

$NaIO_4$

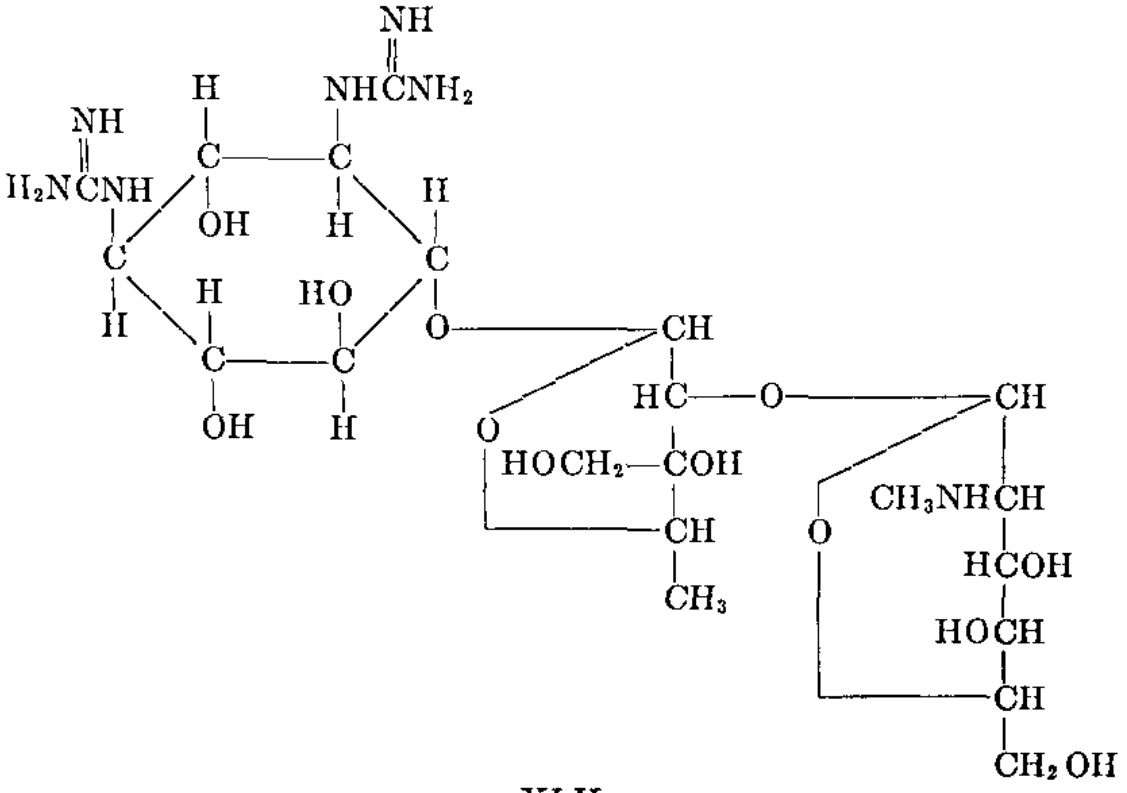


XL.

portion of streptomycin. The formation of maltol from methyl streptobiosaminide dimethyl acetal and from methyl N-acetylstreptobiosaminide showed, as reported by Kuehl, Flynn, Brink, and Folkers (15, 19), that in the formation of maltol from streptomycin and its degradation products, a carbon-carbon rearrangement from a branched to a straight chain must have occurred. It has also been shown that the rearrangement of the streptose moiety to maltol takes place only when the aldehyde group at carbon atom one is glycosidically combined, and when the C-aldehydo group is free or potentially free.

Dihydrostreptomycin

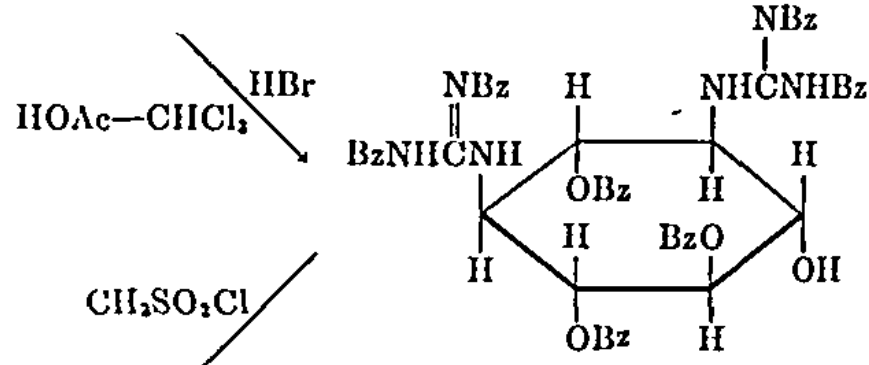
After it was observed that streptomycin possesses a free carbonyl functional group, experiments upon the reduction of this group were undertaken. It was found soon that catalytic hydrogenation using either platinum or nickel catalysts convert streptomycin into dihydrostreptomycin. No structural change other than reduction of the carbonyl group into a carbinol group takes place, and dihydrostreptomycin may be represented by structure XLII.



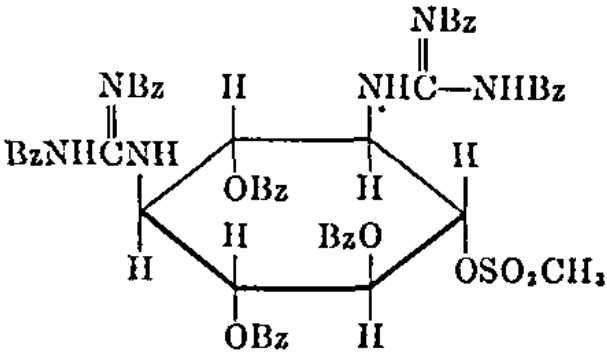
XLII.

Dihydrostreptomycin does not yield maltol when treated with alkali and shows greater alkali stability. It has been demonstrated that dihydrostreptomycin has antibacterial activity comparable to that of streptomycin

Undecabenzoyl-streptomycin

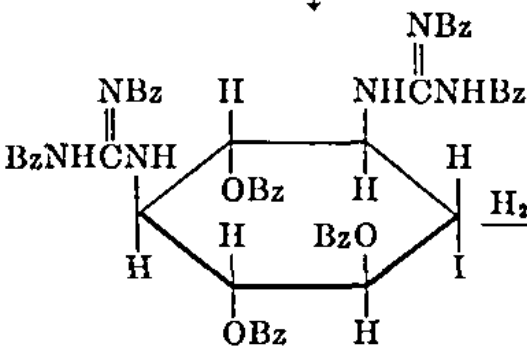


XXXV.
(Bz = benzoyl)



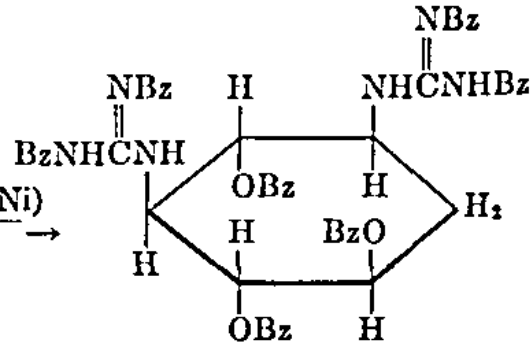
XXXVI.

NaI



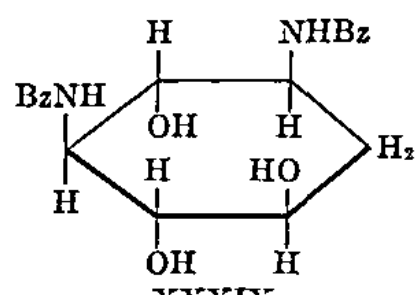
XXXVII.

H_2(Ni) →



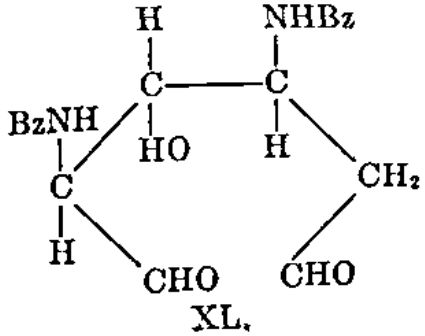
XXXVIII.

(a) hydrolysis
(b) benzoylation
(c) partial debenzoylation

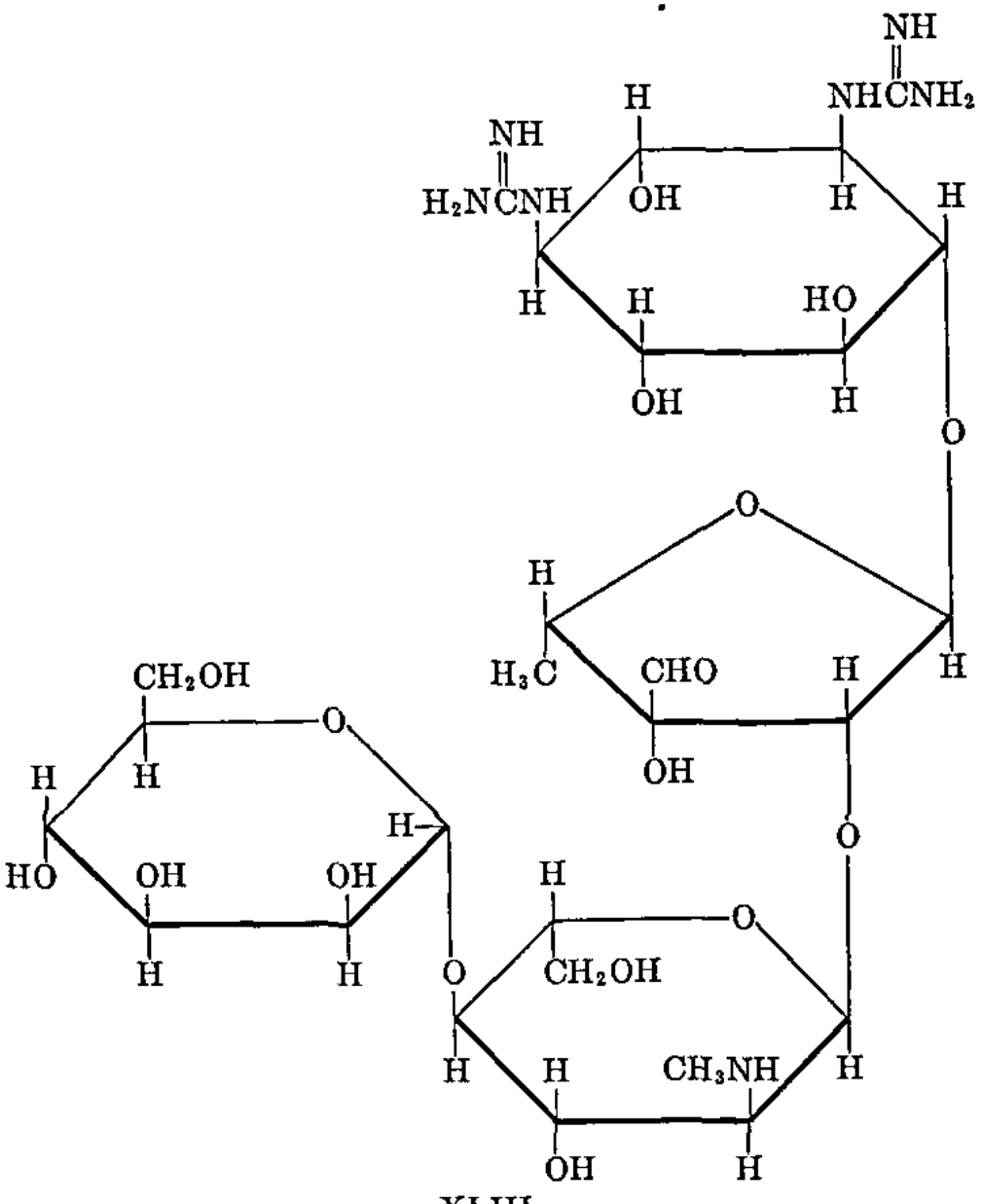


XXXIX.

$NaIO_4$ ←



XL.



XLIII.

established by Fried and Stavely (37) that in mannosidostreptomycin D-mannose is linked glycosidically to N-methyl-L-glucosamine.

The attachment of D-mannose to carbon atom four of N-methyl-L-glucosamine was determined in the classical manner by methylation (37). N-Pentaacetyldihydromannosidostreptomycin was methylated and the product hydrolyzed with acid and then acetylated. A compound identified as triacetyl-3,6-dimethyl-N-methyl-L-glucosamine, XLIV, was isolated. This indicated either C_4 or C_5 as the point of attachment of D-mannose. However, the presence of a pyranose ring in the N-methyl-L-glucosamine portion of mannosidostreptomycin is required for the formation

against *Mycobacterium tuberculosis*. Dihydrostreptomycin is less toxic and the incidence of vestibular disturbances is extremely low; consequently, it is especially desirable for cases requiring relatively high dosages. Because of this marked advantage, dihydrostreptomycin is now being produced commercially.

These results and others, including the usefulness of dihydrostreptomycin in degradative reactions, were reported by Peck, Hoffhine, and Folkers (30), Fried and Wintersteiner (31), Hooper, Klemm, Polglase, and Wolfrom (32), Bartz, Controules, Crooks, and Rebstock (33), and other investigators.

Mannosidostreptomycin

Isolation

Titus and Fried (34) subjected streptomycin concentrates to countercurrent distribution and reported evidence of the presence in the material of a substance other than streptomycin. The second substance was separated from streptomycin chromatographically and was isolated as the crystalline reineckate (35). This substance, which is somewhat less active against various bacteria than streptomycin, was initially named *streptomycin B*. In connection with more recent structural investigations, streptomycin *B* has been renamed *mannosidostreptomycin*. Catalytic hydrogenation of this antibiotic gave dihydromannosidostreptomycin.

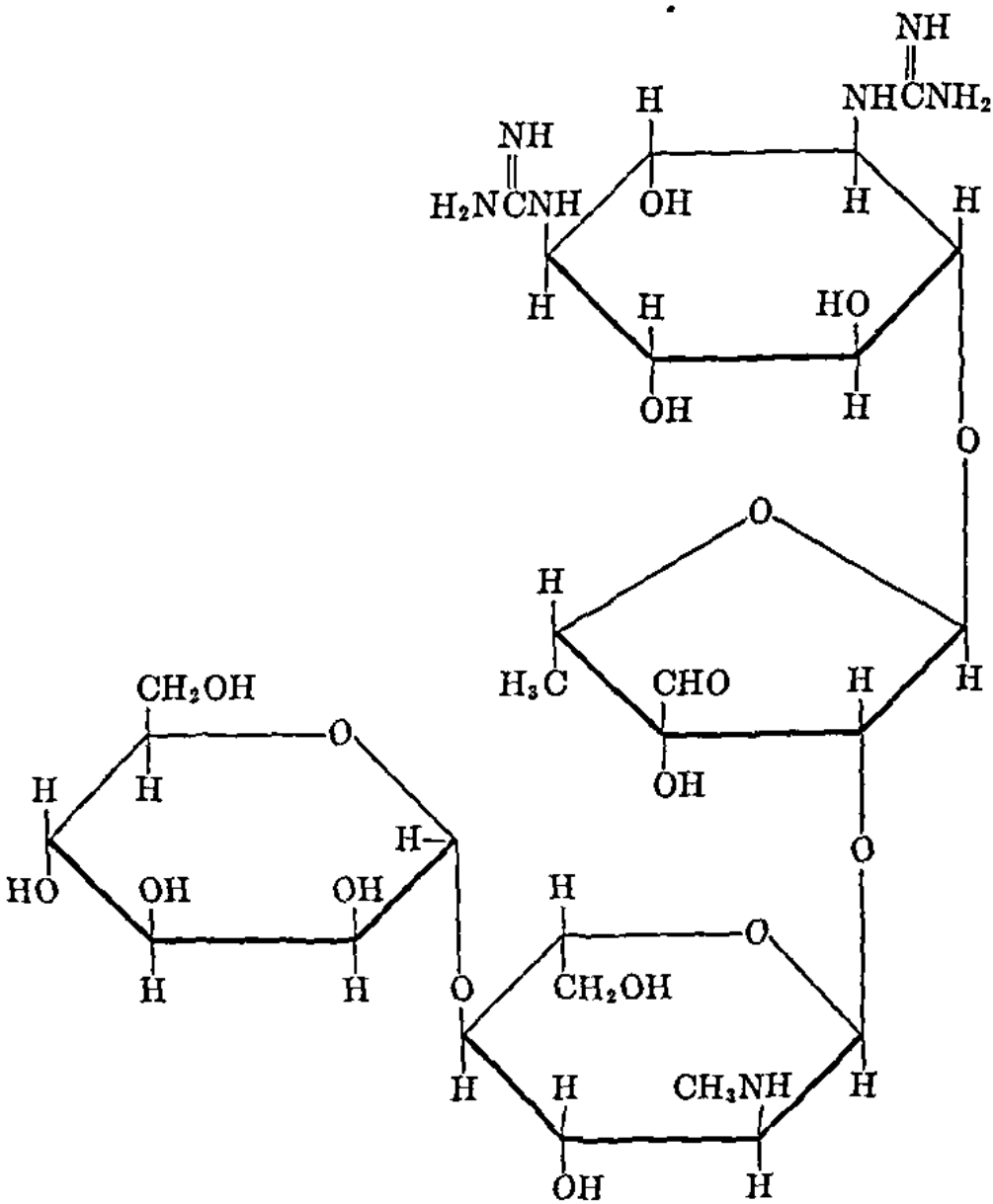
Structure

The structure of mannosidostreptomycin is represented by formula XLIII.

Degradation

When mannosidostreptomycin was treated with methanol and hydrogen chloride, followed by acetylation, Fried and Stavely (36) isolated methyl tetraacetylstreptobiosaminide dimethyl acetal and tetraacetyl-α-methyl D-mannopyranoside. Similar treatment of mannosidostreptomycin with ethyl mercaptan and hydrochloric acid gave, after acetylation, streptidine octaacetate and thio- derivatives of streptobiosamine and D-mannose. Methanolysis of dihydromannosidostreptomycin afforded known derivatives of dihydrostreptobiosamine and of D-mannose.

A mild acid hydrolysis of dihydromannosidostreptomycin gave streptidine and a trisaccharide, isolated as an amorphous monoacetate. The trisaccharide was cleaved either to yield derivatives of dihydrostreptobiosamine and of D-mannose, or to give a disaccharide octaacetate which was further degraded to tetraacetyl-α-methyl-N-methyl-L-glucosaminide and tetraacetyl-α- and tetraacetyl-β-methyl-mannopyranosides. It was thus



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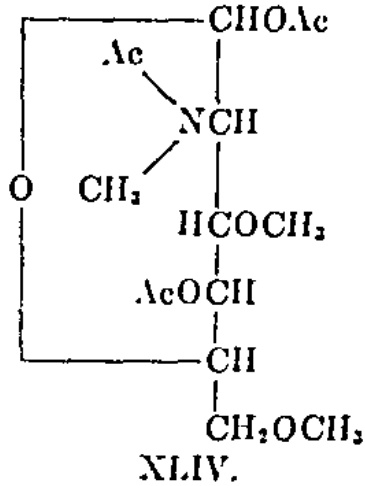
of streptobiosamine derivatives; and thus the D-mannose was placed at position four, rather than five.



XLIV.

Mannosidostreptomycin gave no formaldehyde on periodate oxidation, demonstrating the presence of a pyranoside ring in the mannose portion (37).

Peck, Hoffhine, Gale, and Folkers (38) prepared tetradecabenzoylmannosidostreptomycin and cleaved this compound with hydrogen bromide in chloroform solution to obtain the same heptabenzoylstreptidine (XXXV) which was isolated from a similar degradation of benzoylated streptomycin. It was thus evident that in mannosidostreptomycin the trisaccharide is attached to carbon atom four of streptidine. The knowledge of the structure of mannosidostreptomycin is therefore complete, except for certain points of stereochemistry.

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and very important type of streptomycin which is manufactured by the hydrogenation of streptomycin. This new derivative, dihydrostreptomycin, has already been demonstrated to have a less toxic effect on the eighth nerve, and it is anticipated that it will largely replace streptomycin in the treatment of disease in man in the next few months. As a matter of fact, a marked drop in production of streptomycin from four million grams in September to two million grams in October was the result of industries' rapidly changing their processing over to the manufacture of dihydrostreptomycin. The relatively simple hydrogenation process which results in the modification of the aldehyde group in the streptobiosamine portion of the streptomycin molecule to an alcohol group not only reduces the toxic effect on the eighth nerve, but, in addition, modifies the molecule to such an extent that persons sensitive to streptomycin are not sensitive to dihydrostreptomycin. This is of particular interest, in view of the theoretical considerations that have been given to the possibility that the eighth nerve damage observed with streptomycin may be associated with a sensitivity phenomenon.

The biological methods used for the assay of dihydrostreptomycin are the same as those given for streptomycin in the following pages. Because some organisms are somewhat less susceptible to the action of dihydrostreptomycin than to streptomycin, however, it has been found desirable to specify a single test for the assay of dihydrostreptomycin, namely, the turbidimetric assay using *Kl pneumoniae* as the test organism. At present there are no satisfactory chemical tests for dihydrostreptomycin.

The evidence available indicates that the tubercle bacillus is just as sensitive to the action of dihydrostreptomycin as it is to streptomycin. Certain gram-negative organisms, however, have been found to be somewhat less sensitive to dihydrostreptomycin than to streptomycin. There is no evidence that the bacterial spectrum of dihydrostreptomycin and streptomycin differs significantly, and there is ample evidence that organisms that develop a resistance to streptomycin have similarly developed a resistance to dihydrostreptomycin.

For the present, at least, streptomycin sulfate will continue to be the working standard, and the calcium chloride trihydrochloride double salt of streptomycin the master standard, and dihydrostreptomycin will be labeled on a weight basis in terms of equivalency to streptomycin base. New master and working standards of dihydrostreptomycin, however, are in process of development. The master standard will probably consist of crystalline dihydrostreptomycin hydrochloride; and the working standard, of dihydrostreptomycin sulfate.

A number of methods, each possessing certain advantages and certain limitations, have been developed for the assay of streptomycin. These

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CHAPTER 6

MICROBIOLOGICAL AND CHEMICAL METHODS OF ASSAY FOR STREPTOMYCIN

In January 1947, through mutual agreement between industry and the Food and Drug Administration, Congress was requested to amend the Federal Food, Drug, and Cosmetic Act of June 25, 1938 to provide for certification of batches of drugs composed wholly or partly of any kind of streptomycin or any derivative thereof. This amendment became effective on March 10, 1947, and since that time each batch of streptomycin or streptomycin preparation has been tested for compliance with the regulations promulgated under the amendment. Commercial development of streptomycin proceeded very rapidly because of the valuable experience gained during the last few years in the development of penicillin. In contrast to the latter drug, manufacture of which was initiated as a bottle fermentation process, manufacture of streptomycin was begun through deep tank culture fermentation. In January 1947, prior to the certification of this drug, commercial production had already reached approximately one million grams per month, and by the end of the year had increased to twice that amount. Gradual but steady increases in production continued, until in September 1948 four million grams of streptomycin per month were being produced. It is of interest that a large portion of this was allocated for export.

Although in the early stages of development of streptomycin many problems were encountered similar to those that had been overcome during the development of penicillin, these difficulties with streptomycin were more rapidly solved. For a considerable time it seemed that streptomycin itself was a single chemical entity, but as investigations progressed it became apparent that, like penicillin, it existed in several forms. At present these have been designated as *streptomycin A*, *streptomycin B* (*mannosidostreptomycin*), and a third fraction as yet uncharacterized. The commercial production is composed largely of streptomycin A, of which the potency on a weight basis is approximately five times that of mannosidostreptomycin. Within the last few months considerable interest has developed in a new

PROCEDURE

(a) *Cylinders (cups).* Use stainless steel cylinders with outside diameter 8 mm (±0.1 mm), inside diameter 6 mm (±0.1 mm) and length 10 mm (±0.1 mm).

(b) *Culture medium.* Using ingredients that conform to the standards prescribed by the U.S.P. or N.F., make nutrient agar for the seed and base layers:

Peptone	5.0 gm
Beef extract	3.0 gm
Agar	15.0 gm
Distilled water, q.s.	1000.0 ml
pH 7.8 to 8.0 after sterilization	

(c) *Working standard.* Keep the working standard (obtained from the Food and Drug Administration) constantly in the refrigerator at 15°C (59°F), or below, in tightly stoppered vials, which in turn are kept in larger stoppered tubes containing anhydrous calcium sulfate. Weigh out carefully, in an atmosphere of 50 per cent relative humidity or less, appropriate amounts of the working standard and dilute in 0.05 *M* potassium phosphate buffer (pH 6.0). Keep this stock solution at a temperature of 15°C; do not use it later than 30 days after it is made.

(d) *Standard curve.* Prepare daily in 0.10 *M* potassium phosphate buffer (pH 7.8 to 8.0) a 20 μg/ml solution from the stock solution described above. Transfer to ten 100-ml volumetric flasks, containing the same buffer, the required quantities of this 20 μg/ml solution to give 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4 and 1.5 μg/ml solutions. A total of twenty-seven plates is used in the preparation of the standard curve, three for each solution except the 1.0 μg/ml solution. This last concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 1.0 μg/ml standard and the other three cylinders with the concentration under test. Thus there will be eighty-one 1-μg determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 1.0 μg/ml concentration and the readings of the point tested for each set of three plates and average also all eighty-one readings of the 1.0 μg/ml concentration. This average is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 μg/ml reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8 unit concentration, the average of the eighty-one readings of the 1.0 μg/ml concentration is

methods may vary somewhat in detail because of modifications of techniques introduced by different laboratories. The daily use and evaluation by responsible investigators of certain tests have resulted in the selection of a few that appear to have definite value in assaying the potency of streptomycin preparations. Space does not permit the presentation and discussion of the value and limitations of all these tests, but those that have survived trial are described or mentioned under appropriate groupings.

Methods depending upon observation or measurement of bacterial inhibition are all functions of the biological activity of streptomycin and are dependent upon many variables.

The chemical methods for the assay of streptomycin depend not on biological activity, but rather on chemical structure. Here a high specificity is desirable, since any reaction with similar compounds or with compounds containing similar functional groups serves to reduce materially the range of usefulness of the test.

Bacteriological Methods

The biological methods for determining the potency of streptomycin can be subdivided into three broad groups. One method utilizes modifications of the agar-plate diffusion technique; the second involves turbidimetric measurement of the degree of inhibition of growth of sensitive organisms in the presence of varying concentrations of streptomycin over a few hours' time; and the third makes use of the inhibition of a sensitive organism by dilutions of the streptomycin over a longer period. The last, or serial-dilution method, is the simplest and is widely used in determining streptomycin in body fluids.

The agar-plate diffusion method

The agar-plate diffusion method has been the one most commonly used in the assay of streptomycin to date, although its sensitivity and reproducibility are not quite so satisfactory as those of the turbidimetric method described later in this chapter. The method is very similar to the one used widely for the assay of penicillin. Certain differences inherent in the mode of action, the physical characteristics, and the effects of culture media ingredients on streptomycin result in a method somewhat less accurate than the plate-assay method for penicillin.

The choice of an organism for the plate assay is important, although, theoretically, any sensitive organism could be used. The method that follows employs *B. subtilis* ATCC-6633 and is one of the official tests employed by the Food and Drug Administration in the certification of streptomycin (1).

(i) *Estimation of potency.* Average the zone readings of the standard and average the zone readings of the sample of the three plates used. If the sample gives a larger average zone size than the average of the standard, add the difference between them to the 1.0 μg zone size of the standard curve. If the average sample is lower than the standard value, subtract the difference between them from the 1.0 μg value on the curve. From the curve read the potencies corresponding to these corrected values of zone sizes.

The turbidimetric method

Theoretically, any sensitive organism can be employed, providing it grows rapidly and in such a manner that the degree of turbidity can be correlated with the concentration of streptomycin present in the medium. In the method to be described as illustrative, *Kl. pneumoniae* is used as the test organism. This method is the official method used by the Food and Drug Administration in its streptomycin certification program, and is conducted according to the following procedure for maintaining the test organism, which is *Kl. pneumoniae* (P.C.I. 602) nonencapsulated. Transfer stock cultures every 2 weeks for test purposes. Transfer the organism to fresh agar slants and incubate at 37°C for 6 hours. Suspend the growth from two to three of these slants in sterile distilled water and add approximately 5 ml of culture suspension to each of two Roux bottles containing the same agar. Incubate the bottles for 6 hours at 37°C, harvest the growth and suspend in sufficient sterile distilled water to give a light transmission reading of 80 per cent, using a filter at 6,500 Angstrom units in a photoelectric colorimeter. Keep the resulting suspension of organisms in the refrigerator and use for a period not to exceed 2 weeks.

PROCEDURE

(a) *Employ the following agar:*

Peptone	5.0 gm
Beef extract	3.0 gm
Agar	15.0 gm
Distilled water, q.s.	1000.0 ml

pH 7.8 to 8.0 after sterilization

Prepare a daily inoculum by adding 6.0 ml of this suspension to each 100 ml of a nutrient broth prepared as follows:

Peptone	5.0 gm
Yeast extract	1.5 gm
Beef extract	1.5 gm
Sodium chloride	3.5 gm
Glucose	1.0 gm
Dipotassium phosphate	3.68 gm

16.5 mm and the average of the 1.0 μg/ml concentration of this set of three plates is 16.3 mm, the correction is 0.2 mm. If the average reading of the 0.8 μg/ml concentration of these same three plates is 15.9 mm, the corrected value is then 16.1 mm. Plot these corrected values, including the average of the 1.0 μg/ml concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points. The ten points selected to determine the curve are arbitrary and should be so chosen that the limits of the curve will fill the needs of the laboratory. However, the potency of the sample under test should fall in the interval from 60 per cent to 150 per cent of the correction point of the standard curve.

(e) *Preparation of sample.* Dissolve volumetrically in sterile distilled water the sample to be tested to make a convenient stock solution. Further dilute this solution volumetrically to contain 100 μg of streptomycin base (estimated) per milliliter. Transfer 1.0 ml of this 100 μg (estimated)/ml solution to a 100-ml flask and make up to volume with 0.10 *M* potassium phosphate buffer (pH 7.8 to 8.0). Use this last dilution in the assay for potency.

(f) *Preparation of spore suspension.* The test organism is *B. subtilis* (American Type Culture Collection 6633). Maintain the test organism on nutrient agar prepared as described above (b). Prepare a spore suspension by growing the organism in Roux bottles on agar of this same composition for 1 week at 37°C. Suspend the spores in sterile distilled water, and heat for 30 minutes at 65°C. Wash the spore suspension three times with sterile distilled water, heat again for 30 minutes at 65°C, and resuspend in sterile distilled water. Maintain the spore suspension at approximately 15°C. Determine by appropriate tests the quantity of spore suspension to be added to each 100 ml of agar for the secondary layer that will give sharp clear zones of inhibition.

(g) *Preparation of plates.* Add 21 ml of agar described in paragraph (b) to each Petri dish (20 by 100 mm) Melt the agar to be used for the secondary layer, cool to 55° to 60°C, and add the spore suspension prepared in (f). Mix thoroughly and add 4 ml to each of the plates containing the 21 ml of the uninoculated agar. Tilt the plates back and forth to spread the inoculated agar evenly over the surface. Refrigerate until ready to add streptomycin (at least 1 hour).

(h) *Plate assay.* Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on 2.8-cm radius. Use three plates for each sample. Fill three cylinders on each plate with the 1.0 μg/ml standard and three cylinders with the 1.0 μg/ml (estimated) sample; alternating standard and sample. Incubate the plates for 16 to 18 hours at 37°C and measure the diameter of each circle of inhibition.

[see table in paragraph (b)], the latter concentrations for each concentration level of the standard may be expressed as percentages and substituted on the abscissa of the standard curve. If this is done, the percentage potency of the sample under test may be read directly from the standard curve.

The turbidimetric assay has the advantage of being a rapid test and in capable hands appears more accurate and reproducible than the plate technique. The inherent disadvantages are the requirement of relatively larger amounts of the sample under test and the necessity of using aseptic technique and sterile material.

The serial dilution method

The serial dilution method of assay for streptomycin is the simplest of the commonly employed techniques, requiring only a sensitive organism, a sterile preparation, and a minimum of equipment. The *Kl. pneumoniae* method described by Donovick, Hamre, Kavanagh, and Rake (2) illustrates well this type assay.

Grow *Kl. pneumoniae* ATCC-9947 in yeast-beef broth and prepare a 1:100,000 dilution with 0.75 per cent tryptone water (pH 8.5). Distribute 2.0-ml amounts of this suspension in a series of ten test tubes.

Dilute the sample to contain an estimated potency of 2.0 μg/ml with distilled water. Using a chemically clean Kahn pipette, add the following amounts to one of the series: 0.1, 0.080, 0.077, 0.068, 0.052, 0.046, 0.040, 0.035, and 0.030 ml. Include one or more series prepared exactly as above with standard streptomycin preparation in each day's run. Incubate at 37°C overnight and record the degree of growth in each tube, considering the one showing plus-minus growth as the endpoint. The activity of the unknown specimen is then calculated according to the following formula:

$$\frac{Ax}{Ac} = \frac{Vc}{Vx}$$

in which:

Ax is the unknown in micrograms per ml,
Ac is the standard in micrograms per ml,
Vc is the volume of the standard causing inhibition,
Vx is the volume of the unknown causing inhibition.

The disadvantages in a serial dilution assay method for determining the potency of streptomycin preparations are numerous. Although all are extremely simple techniques and require a minimum of apparatus, they are exacting and time-consuming. Sterile techniques are essential, and the sensitivity of the organism may be such that large relative changes in concentration are required to produce a visible change in growth. The exact point at which inhibition is complete is often difficult to determine.

Potassium dihydrogen phosphate	1.32 gm
Distilled water, q.s.	1000.0 ml
pH 7.0 after sterilization	

(b) *Working standard solutions.* Add the following amounts of a 1,000 μg/ml solution prepared from the stock solution described in (c) *(Streptomycin Plate Assay)* to 100-ml volumetric flasks containing sterile distilled water and bring to volume to give the working stock solutions tabulated below. These nine flasks are well stoppered and maintained at approximately 15°C for 1 month. Prepare final dilutions daily by adding 2.1 ml of each of these nine working stock solutions to 4.8 ml of sterile distilled water. Add 1.0 ml of each final dilution to each of six 14 by 124 mm tubes (total fifty-four tubes). Add 9.0 ml of inoculated broth described above to each tube and place immediately in a 37°C water bath for 4 hours. The final concentration of streptomycin per milliliter of broth is also included in the following tabulation:

AMOUNT OF STANDARD SOLUTION (1,000 μG/ML)	WORKING CONCENTRATION/ML (ALSO PERCENTAGE CONCENTRATION)	FINAL CONCENTRATION (μG/ML) AFTER ADDITION OF DISTILLED WATER AND BROTH
ml	*μg*	*μg*
6.0	60	1.8
7.0	70	2.1
8.0	80	2.4
9.0	90	2.7
10.0	100	3.0
11.0	110	3.3
12.0	120	3.6
13.0	130	3.9
14.0	140	4.2

(c) *Preparation of sample.* Dilute the sample under test with sterile distilled water to contain 100 μg/ml (estimated). To 2.1 ml of the sample add 4.8 ml of sterile distilled water, add 1.0 ml of this dilution to each of six 14 by 124 mm tubes. Add 9.0 ml of the inoculated broth described in paragraph (a) to each tube and place immediately in 37°C water bath for 4 hours. A control tube containing 1.0 ml of distilled water and 9.0 ml of the inoculated broth is similarly incubated. After incubation, add 4 drops of formalin to each tube, and read the light transmission in a photoelectric colorimeter, using a broad-band filter having a wavelength of 5,300 Angstrom units.

(d) *Estimation of potency* Average the light transmission readings for each concentration of the standard. Plot these values on cross-section paper, employing average light transmission readings as the ordinate, and streptomycin concentration per milliliter of broth as the abscissa. Prepare a standard curve by connecting successive points with a straight edge.

example the 10 μg standard produced inhibition in the fifth tube. ently 5.0 μg/ml would have produced inhibition in the fourth tube n the fifth, 2 5 μg/ml would have produced inhibition in the third t not in the fourth, and 20 μg/ml should have produced inhibition sixth tube, and 40 μg/ml in the seventh.

readily apparent that this method measures relatively broad changes ncentration. For clinical use, where the information ordinarily de- is whether the agent is present in quantities desired for treatment of condition, the test is adequate.

Miscellaneous methods

The miscellaneous methods usually are employed only in the determination of streptomycin in blood serum or other body fluids and follow one of the groups already illustrated in detail. Each has its individual advantages and disadvantages. In general, these methods are limited to the plate and serial dilution techniques because of the growth-promoting effect of serum on organism used in the turbidimetric techniques.

The plate method described earlier in this chapter can be adapted to the assay of streptomycin in body fluids by preparing the standard in normal serum, but its sensitivity is not so great as some of the methods designed specifically for use with body fluids.

Both plate and serial dilution methods have been described and used widely in the assay of streptomycin in body fluids (4, 5, 6, 7, 8, 9, 10).

Chemical Methods

The chemical methods currently employed in the assay of streptomycin depend on: (a) formation of an easily identified substance, maltol, from the biologically active portion of the streptomycin molecule; (b) formation of identifiable complexes by interaction of the potential streptose aldehydic group of streptomycin with appropriate reagents and; (c) interaction of a substance with the biologically inactive portion of the molecule, to form a readily measurable substance.

The first and second methods have the advantage of measuring only the active streptomycin, but they cannot be applied to dihydrostreptomycin, since the potential aldehydic group in this instance has been converted to an alcoholic group by catalytic hydrogenation. The last method, which utilizes a portion of the molecule in which the activity does not reside, can be applied to dihydrostreptomycin, but its usefulness is limited somewhat by the fact that certain of the breakdown products may also give the reaction, resulting in a falsely high value. The last method is one that usually involves the streptidine residue which contains the guanido groupings.

The twofold serial dilution method

This method of assay is used almost entirely for determining the concentration of streptomycin in clinical specimens where only a rough estimation is required. A minimum of technique and of laboratory equipment is necessary for the conduct of this type test. Although any sensitive organism can theoretically be used, those which grow with a pellicle or produce a granular growth are preferable because of the ease with which the endpoint can be determined. The organism must not be inhibited or unduly affected by the concentrations of serum or body fluids employed. The method described by Price, Nielsen, and Welch (3) is representative:

B. circulans, the test organism employed, grows readily at temperatures of 30–37°C, forming flocculcs which make the endpoint easy to determine. The organism, which is sensitive to 0.15 μg/ml of streptomycin, grows well in ordinary media and can be preserved under refrigeration for 1 month with no loss in sensitivity. Because of the interfering effects of various compounds on the action of streptomycin, a simple medium consisting of peptone 1 per cent, beef extract 0.5 per cent, and sodium chloride 0.25 per cent is used for the assay. The medium is adjusted to a pH of 7.8 to 8.0 with NaOH, since streptomycin exerts its greatest activity at this hydrogen-ion concentration.

In conducting the test, 0.5-ml amounts of the medium are added to Wassermann tubes, and serial dilutions by halves are made through the desired number of tubes, using 0.5 ml of the fluid under test and carrying 0.5-ml amounts through the series, discarding 0.5 ml from the last tube. A 0.5-ml amount of the fluid under test is added to an empty tube and placed first in the series. A standard series is prepared exactly as above, a solution containing 10 μg/ml being used. This standard can be prepared in water or in serum, since no differences are detectable in either fluid. A 1:100 dilution of the test organism is prepared from the assay broth, and 1.5 ml is added to each tube. After overnight incubation at 37°C, the tubes are read and the endpoint taken as the last tube in each series showing no growth. The concentration of the streptomycin in the unknown is then determined by comparing the endpoints observed with the standard series. Following is an example:

FLUID	TUBE NUMBERS						
	1	2	3	4	5	6	7
Standard.	0	0	0	0	0	+	+
Serum .	0	0	0	+	+	+	+
Urine 1:50	0	0	0	0	+	+	+

In this example the 10 μg standard produced inhibition in the fifth tube. Consequently 5.0 μg/ml would have produced inhibition in the fourth tube but not in the fifth, 2.5 μg/ml would have produced inhibition in the third tube but not in the fourth, and 20 μg/ml should have produced inhibition in the sixth tube, and 40 μg/ml in the seventh.

It is readily apparent that this method measures relatively broad changes in concentration. For clinical use, where the information ordinarily desired is whether the agent is present in quantities desired for treatment of the condition, the test is adequate.

Miscellaneous methods

The miscellaneous methods usually are employed only in the determination of streptomycin in blood serum or other body fluids and follow one of the groups already illustrated in detail. Each has its individual advantages and disadvantages. In general, these methods are limited to the plate and serial dilution techniques because of the growth-promoting effect of serum on organism used in the turbidimetric techniques.

The plate method described earlier in this chapter can be adapted to the assay of streptomycin in body fluids by preparing the standard in normal serum, but its sensitivity is not so great as some of the methods designed specifically for use with body fluids.

Both plate and serial dilution methods have been described and used widely in the assay of streptomycin in body fluids (4, 5, 6, 7, 8, 9, 10).

Chemical Methods

The chemical methods currently employed in the assay of streptomycin depend on: (a) formation of an easily identified substance, maltol, from the biologically active portion of the streptomycin molecule; (b) formation of identifiable complexes by interaction of the potential streptose aldehydic group of streptomycin with appropriate reagents and; (c) interaction of a substance with the biologically inactive portion of the molecule, to form a readily measurable substance.

The first and second methods have the advantage of measuring only the active streptomycin, but they cannot be applied to dihydrostreptomycin, since the potential aldehydic group in this instance has been converted to an alcoholic group by catalytic hydrogenation. The last method, which utilizes a portion of the molecule in which the activity does not reside, can be applied to dihydrostreptomycin, but its usefulness is limited somewhat by the fact that certain of the breakdown products may also give the reaction, resulting in a falsely high value. The last method is one that usually involves the streptidine residue which contains the guanido groupings.

The maltol method

This method depends on the fact that streptomycin, when heated in the presence of dilute alkali, forms maltol (2-methoxy-3-hydroxy-gamma-pyrone) (11). Maltol is formed from the biologically active portion of the streptomycin molecule and shows characteristic absorption in the ultraviolet region of the spectrum. It remains stable in acid solutions and gives colors with ferric ions. When ferric ammonium sulfate is used in developing the color, a sensitivity of 500 to 2,500 μg of streptomycin is found. When the phenol reagent of Folin and Ciocalteau (12) is substituted in the method described by Boxer, Jelinek and Leghorn (13), the test becomes sensitive to 20 to 250 μg of streptomycin.

Maltol is produced, under the conditions of this test, by streptomycin but not by dihydrostreptomycin, N-methyl-L-glucosamine, streptidine, or streptobiosamine. Maltol is formed from the streptose (14) portion of the intact streptomycin molecule.

This method, which can be used for determining streptomycin in body fluids as well as in aqueous or methanolic solutions, is conducted according to the following procedure:

Reagents:

1 *N* NaOH
2 *N* NaOH
20 per cent sodium carbonate solution
1 per cent ferric ammonium sulfate in 0.75 *N* sulfuric acid
Phenol reagent of Folin and Ciocalteau.

Procedure. Add 1.0 ml of 2 *N* NaOH to 5.0 ml of the streptomycin solution being tested, and immerse the test tube in a boiling water bath for 3 minutes. Cool in water for 3 minutes. If the amount of streptomycin present is estimated at 20 to 250 μg, the color is developed with the phenol reagent; if the amount of streptomycin is estimated at 500 to 2,500 μg, the ferric ammonium sulfate solution is used.

When the phenol reagent is to be employed, 1.0 ml is added drop-wise to the cooled alkaline solution and mixed thoroughly. After 1 to 2 minutes, 3.0 ml of the sodium carbonate solution is added and the tube shaken thoroughly. Let stand 10 minutes and determine the light transmission in a photoelectric colorimeter with a 6,600 Angstrom unit filter. A blank is prepared and examined simultaneously.

When the ferric ammonium sulfate solution is employed, 4.0 ml of the reagent is added to the cooled alkaline mixture and the tube allowed to sit for 10 minutes while the purple-red color develops. The light transmission is then determined with a 5,400 Angstrom unit filter. The light transmission of a blank prepared as above is essentially the same as water when the

ferric ammonium sulfate reagent is used, and water can be substituted for the blank.

Small quantities of material are always present in clinical specimens which react with the phenol reagent. These usually account for 2 to 5 per cent of the final color obtained in the maltol procedure. To determine the value of these interfering substances, an additional determination is carried out on 5 to 10 times the amount of the sample in exactly the manner described, with the exception that the heating period with the alkali is omitted. Since the formation of maltol from streptomycin occurs at an appreciable rate even at room temperature, the phenol reagent must be added immediately after addition of the alkali.

Methanolic solutions of streptomycin can be assayed by this method with minor modifications. Four milliliters of the solution to be assayed is placed in a 10-ml graduated cylinder, 2.0 ml of 1 *N* NaOH is added, and the cylinder immersed in a water bath at 65°C for 1 minute. After cooling, the ferric ammonium sulfate reagent is added to the 10-ml mark and the color measured after 10 minutes with a 6,600 Angstrom unit filter.

A standard preparation of streptomycin is used in establishing a curve by the above procedures. Since streptomycin is a hygroscopic salt, two samples are weighed. One is dried for 3 hours at 100°C *in vacuo*, a process that results in a loss in potency, and the other is used in determining the standard curve after the appropriate corrections for moisture have been made.

Marshall's semicarbazide method

Marshall's semicarbazide method for the assay of streptomycin is based upon the interreaction of the carbonyl or potential aldehyde group in the streptomycin molecule with a colored semicarbazide to form a colored complex which can be determined colorimetrically. The substance used in the Marshall (15) procedures is 4-(-4(p-chlorophenylazo)-1-naphthyl) semicarbazide. Semicarbazide is rather insoluble in water, but when it is dissolved in methyl cellosolve it reacts readily with streptomycin to form the colored derivative. The peak absorption of this derivative is about 580 millimicrons when examined in the Beckman spectrophotometer. This method has been adapted for assay in blood serum, and the modified maltol method can be used in determining streptomycin in the urine. The procedures are conducted as follows:

A. DETERMINATION OF STREPTOMYCIN IN SERUM

Reagents:

Semicarbazide solution: Dissolve 0.135 gm of semicarbazide in 50 ml of redistilled methyl cellosolve by warming to 50°C. Dissolve 2.66 gm of sodium acetate

trihydrate in the solution so obtained and add 8.3 ml of glacial acetic acid. Bring the mixture to room temperature and dilute to the 100-ml mark with methyl cellosolve.
Chloroform
Concentrated hydrochloric acid
Trichloroacetic acid: 15 gm made up to a volume of 100 ml in water.

Procedure. Add 3 ml of water and 1 ml of trichloracetic acid solution to 1 ml of plasma, let stand 20 minutes and centrifuge. Add 3 ml of the plasma centrifugate to 3.0 ml of the semicarbazide solution contained in a glass-stoppered centrifuge tube graduated at 3.5 ml. Heat the tube for 15 minutes in a boiling water bath and cool by immersion in ice water. Add 10 ml of chloroform and shake the stoppered tube at least 100 times. After separation, remove the chloroform with a capillary pipette or with a syringe and a long needle. Repeat the extraction with two or more 10-ml portions of chloroform. After removal of the final chloroform layer, add water to the 3.5 ml mark. Treat 3.0 ml of this with 3.0 ml of concentrated HCl and allow to come to room temperature. Determine the intensity of the blue color in a photoelectric colorimeter equipped with a 5,800 Angstrom filter. The colorimeter is set at zero with a blank made by adding 3 ml of concentrated HCl to an equal volume of a solution obtained by dissolving 30 ml of methyl cellosolve in 100 ml of water.

B. DETERMINATION OF STREPTOMYCIN IN URINE

Reagents:

2.5 *N* NaOH
4 *N* HCl
Chloroform
Ferric nitrate solution: 0.5 gm ferric nitrate monohydrate in 100 ml of 0.035 *N* nitricacid. Dilute to 400 ml final volume.

Procedure. Dilute the urine in such a manner that it contains not more than an estimated 2,000 μg of streptomycin in 3.0 ml. Place 3.0 ml of the diluted urine and 0.7 ml of 2.5 *N* NaOH in a glass-stoppered 125 ml pyrex bottle and immerse in boiling water for 5 minutes. Cool in ice water and add 0.5 ml 4 *N* HCl and 60 ml of chloroform. Shake for 5 minutes. Withdraw 50 ml of the chloroform phase and shake for 10 minutes with 10 ml of the iron reagent. Withdraw a portion of the aqueous phase and determine the relative optical density in a photoelectric colorimeter equipped with a filter at 545 millimicrons. Correct the colorimeter reading for that given by a blank on each sample of urine used. The blank is prepared by treating a portion of each urine sample in the fashion described, but no sodium hydroxide or heat is used.

The oxidized nitroprusside method

The oxidized nitroprusside method depends upon the reaction between oxidized nitroprusside and guanidine groups. Two guanido groups are present in the streptomycin molecule as portions of the streptidine moiety. Unfortunately, this portion of the molecule is not the one in which activity resides and the reaction may be given by streptidine. The latter compound is extremely insoluble, however, and when concentrated solutions of streptomycin in the presence of degradation products are made up, the streptidine portion precipitates or fails to go into solution. This assay is conducted according to the procedure described by Sullivan and Hilmer (16):

Reagent:

Oxidized nitroprusside (Weber's modification):	
10 per cent Sodium nitroprusside	1 ml
10 per cent Potassium ferricyanide	1 ml
10 per cent Sodium hydroxide	1 ml
Distilled water	9 ml

Mix and let stand 30 minutes before use.

Procedure. Place 1.0 ml of a solution containing 1,000 to 3,000 μg of streptomycin in a test tube and add 1.0 ml of the freshly prepared nitroprusside reagent and 3.0 ml of distilled water. Allow to stand at room temperature for 10 minutes. Determine the light transmission in a photoelectric colorimeter using a broad-band filter at 5,400 Angstrom units. Prepare a standard curve using streptomycin standard at 1,000, 1,500, 2,000, 2,500, and 3,000 μg/ml. Determine the concentration of streptomycin in the unknown by locating the point on the standard curve which corresponds to the observed light transmission.

This test is given by guanidines also.

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Section II

ANTIBACTERIAL AND PHARMACOLOGIC PROPERTIES OF STREPTOMYCIN

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CHAPTER 7

ACTION OF STREPTOMYCIN ON MICROORGANISMS IN VITRO

The outstanding feature of the action of streptomycin as observed *in vitro* is its ability to inhibit the growth of certain microorganisms. The degree of inhibition of growth not only is directly proportional to the concentration of streptomycin, but depends upon the type and number of microorganisms employed and upon the environmental conditions under which the exposure to streptomycin takes place.

This antimicrobial action of streptomycin *in vitro* has been described as *bacteriostatic* in low concentrations and as *bactericidal* in high concentrations (1, 2), the former term being used to indicate only the prevention of multiplication, and the latter to indicate death of the exposed microorganisms. Though it is not within the scope of this chapter to discuss the mode of action of streptomycin, the above explanation would appear to be oversimplified. In fact, very low concentrations of streptomycin actually may stimulate growth of bacteria (3), even though growth is inhibited by higher concentrations. Furthermore, other factors may influence the degree of bacteriostatic or bactericidal action. These are (a) the type of microorganism, (b) the nature of the medium, (c) the time of exposure, (d) the hydrogen-ion concentration, (e) the number of organisms employed, and (f) the temperature. It might be possible for a given concentration of streptomycin to be either bacteriostatic or germicidal, depending upon the organism used and the conditions of the test.

The effectiveness of streptomycin *in vitro* for the inhibition of growth of microorganisms is usually expressed in terms of the smallest amount per milliliter of medium which completely prevents growth. Such concentrations of streptomycin are by custom referred to as bacteriostatic and furnish a basis for a comparison of the activity of streptomycin for different microorganisms. Furthermore, these minimal inhibiting concentrations may indicate the effectiveness of streptomycin for the treatment of infection.

Bacteriostatic Activity of Streptomycin

Table 7 shows the bacteriostatic activity of streptomycin in micrograms or units per milliliter for a wide variety of microorganisms. An attempt has been made to include in this table all microorganisms that have been tested *in vitro* for their sensitivity to streptomycin. Though this goal probably has not been reached, certainly the majority of such organisms have been included.

Members of two classes of microorganisms have been tested, the Schizomycetes and the Eumycetes, as well as some members of the phylum Protozoa. Insofar as we have been able to determine, organisms sensitive to streptomycin have been reported only among the Schizomycetes.

Among the eight orders into which the Schizomycetes have been divided, there are only two, the Chlamydobacteriales and the Myxobacteriales, no members of which were found to have been tested for their sensitivity to streptomycin. A few of the Virales have been tested, but no streptomycin-sensitive members have been encountered. The order, Rickettsiales, contains only one family, the *Rickettsiaceae*, some of which when grown in yolk sac cultures are inhibited slightly by streptomycin. No *in vitro* tests have been reported on any of the Borrelomycetales, but some are susceptible to the action of streptomycin *in vivo*. The Eubacteriales, Actinomycetales, and Spirochaetales contain the majority of susceptible organisms, most of these being found within the order Eubacteriales.

The order Eubacteriales is divided into thirteen families, of which, insofar as we have been able to find, only the *Nitrobacteriaceae* and *Azotobacteriaceae* have not been tested for their sensitivity to streptomycin. At least a few members of all the remaining families have been tested. Although, as discussed below, many of the data on the sensitivity of bacteria to streptomycin *in vitro* are difficult to compare and interpret, the following broad generalizations appear to be valid.

In some families, all the genera tested are sensitive to streptomycin; in other families, only some genera are sensitive. Within genera, there is considerable variation in susceptibility to streptomycin between species; within species, there is frequently a marked difference in the susceptibility of individual strains of bacteria.

It would appear, however, that within certain families some genera are more uniformly susceptible than others. These are the genera *Brucella*, *Hemophilus*, and *Pasteurella* within the family *Parvobacteriaceae*, the genus *Bacillus* in the *Bacillaceae*, the genera *Erysipelothrix* and *Listeria* in the family *Corynebacteriaceae*, and the genera *Shigella* and *Klebsiella* in the family *Enterobacteriaceae*.

In the family Actinomycetales, the *Mycobacteriaceae*, with the excep-

TABLE 7

Sensitivity in vitro of microorganisms to streptomycin

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Class: Schizomycetes†		
Order: Eubacteriales		
Family: Pseudomonadaceae		
Pseudomonas aeruginosa (*B. pyocaneus*)	6, 60, 73, 144, 190, 274, 309, 360, 446, 469, 496, 525, 602, 653, 656, 913, 948, 1073, 1147	0.1 –500.0
Pseudomonas fluorescens	52, 255	4.0 – 12.5
Pseudomonas incognita, *Pseudomonas jaegeri*, *Pseudomonas pierantonii*	469	7.0 –500.0
Vibrio cholerae (*Vibrio comma*)	52, 125, 274 A	5.0 –500.0
Xanthomonas pruni	52	0.25
Family: Rhizobiaceae		
Chromobacterium ianthinum	469	50.0
Chromobacterium violaceum	360	4.0
Family: Micrococcaceae		
Micrococcus aerogenes	469	0.5 – 75.0
Micrococcus aurantiacus	469	10.0 –250.0
Micrococcus citreus (*Staphylococcus citreus*)	469	0.5 – 75.0
Micrococcus pyogenes, var. *albus* (*Staphylococcus albus*)	60, 186, 360	0.03 –256.0
Micrococcus pyogenes, var. *aureus* (*Staphylococcus aureus*)	6, 60, 138, 150, 151, 360, 469, 495, 496, 647, 656, 1147	0.03 –>256.0
Micrococcus ureae	469	10.0 –250.0
Micrococcus sp.	60, 360	0.5 – 2.0
Sarcina lutea	1	0.25

* Numbered references listed will be found in "The Literature on Streptomycin, 1944–1948," Rutgers University Press, New Brunswick, N. J. References designated by letters will be found appended to the table.

† The nomenclature used for the Schizomycetes follows that of Bergey's Manual of Determinative Bacteriology, 6th Edition, 1948. Where other names are more commonly employed than those recommended by Bergey, these also have been included. Species of bacteria not listed in Bergey's Manual have been included as named in the original publications.

TABLE 7—*Continued*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Family: Neisseriaceae		
Neisseria catarrhalis	360	1.0 - 4.0
Neisseria gonorrhoeae	52, 75, 151, 274	1.0 - 40.0
Neisseria meningitidis (*Neisseria intracellularis*)	52, 75	1.0 - 40 0
Veillonella gazogenes	B	10.0
Family: Lactobacteriaceae		
Diplococcus pneumoniae	6, 360, 469, 495, 645	0.5 - 50.0
Enterococcus	496	0.2 -<100
Streptococcus sp., alpha hemolytic	6, 60, 148, 360, 496	0.06 ->256.0
Streptococcus sp , beta hemolytic	60, 150, 151, 469, 647	0.06 ->256.0
Streptococcus sp., gamma type	309, 360, 647	0.5 -128.0
Streptococcus agalactae	1147	10.0
Streptococcus canis	1147	10.0
Streptococcus dysgalactae (*Streptococcus equisimilis*)	1147	5.0
Streptococcus faecalis	144, 309, 469	1 0 -100.0
Streptococcus gallinarum	1147	10.0
Streptococcus lactis	6	>16.0
Streptococcus pyogenes	6	2.0 ->16 0
Streptococcus uberis	1147	10.0
Streptococcus zooepidemicus	1147	10.0
Family Corynebacteriaceae		
Corynebacterium diphtheriae	52, 274, 360, 645, 957	0.375-200.0
Corynebacterium pseudotuberculosis	1147	1.0
Corynebacterium pyogenes	1147	0.25 - 10.0
Corynebacterium renale	1147	0.5 - 2.5
Corynebacterium ulcerogenes	469	1.0 -250.0
Corynebacterium xerose	469	1.0 -250.0
Diphtheroids	60, 360	0 03 ->256 0
Erysipelothrix muriseptica	4	2.5
Erysipelothrix rhusiopathiae	1147	10.0
Listeria monocytogenes (*Listerella monocytogenes*)	1, 52, 1034, 1147	1 0 - 2.5
Family· Achromobacteriaceae		
Alcaligenes faecalis	309, 360, 446, 651	2 0 - 64.0
Family: Enterobacteriaceae		
Aerobacter aerogenes	60, 144, 190, 274, 309, 469, 495, 1147	0.3 ->256 0
Aerobacter cloacae	197	0.5

TABLE 7—*Continued*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Family: Enterobacteriaceae (cont'd)		
Escherichia coli	6, 58, 60, 73, 138, 144, 150, 190, 197, 274, 309, 360, 446, 469, 645, 647, 653, 656, 948, 1147	0.015–>256.0
Escherichia communior	52, 255	1.0 – 8.0
Escherichia freundii	469	0 3 – 50.0
Escherichia neapolitana	255	8.0
Klebsiella ozaenae	9, 52	0.15 – 1.5
Klebsiella pneumoniae	9, 138, 150, 190, 309, 360, 446, 645, 656, 948, 1147	0 055–128.0
Klebsiella pneumoniae, A	151, 360, 495	0.8 –128.0
Klebsiella pneumoniae, B	360	2.0 –128 0
Klebsiella pneumoniae, C	495	0.8
Paracolobactrum sp. (*Paracolon bacilli*)	144, 190, 360, 446	2.0 –128.0
Proteus mirabilis	360, 469	3 0 – 64.0
Proteus morganii	73, 309, 360, 469, 525, 645	1.0 –128 0
Proteus vulgaris	6, 60, 190, 360, 446, 469, 525, 645, 647, 653, 948, 1147	1.0 –128.0
Salmonella anatis	58	8.0 – 32 0
Salmonella choleraesuis	58, 1147	4.0 – 32 0
Salmonella choleraesuis, var. *kunzendorf*	58	4.0 – 16.0
Salmonella enteritidis	6, 58, 360, 417, 1147	0.004– 32.0
Salmonella gallinarum	58	2 0 – 16.0
Salmonella hirschfeldii (Para C)	58, 1147	8.0 –>10 0
Salmonella morbificans	417	0.004– 0 064
Salmonella paratyphi (Para A)	58, 360, 645	2 0 – 8 0
Salmonella pullorum	58, 360, 1147	1.0 – 16.0
Salmonella schottmülleri (Para B)	6, 58, 360, 417, 645	0.004– 32.0
Salmonella typhosa (*Eberthella typhosa*)	6, 58, 73, 274, 360, 417, 645	0 004– 20 0
Salmonella typhimurium (*Salmonella aertrycke*)	6, 58, 274, 360, 417, 1147	0.004– 32.0
Salmonella typhisuis	1147	10.0

TABLE 7—*Continued*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Family: Enterobacteriaceae (cont'd)		
Salmonella species		
Type Bareilly	58	8.0 – 32.0
Type Derby	58, 417	0.004– 16.0
Type Give	58	8.0 – 32.0
Type Manhattan	417	0.004– 0.064
Type Minnesota	417	0 004– 0.064
Type Montevideo	58, 417	0.004– 32.0
Type Newington	58, 417	0.004– 32.0
Type Newport	58, 417	0.004– 16.0
Type Oranienberg	58, 417	0 004– 16 0
Type Oregon	417	0.004– 0 064
Type Saint Paul	417	0.004– 0.064
Type Senftenberg	58	4.0 – 32.0
Type Tennessee	417	0.004– 0.064
Type Thompson	417	0.004– 0.064
Salmonella species.		
60 types, unspecified	341	4.0 – 8.0
Serratia indica	360	8.0
Serratia marcescens	52, 360	1.0 – 64.0
Shigella ambigua	110	3.0 – 7.0
Shigella dysenteriae	110, 360, 645	2.0 – 8.0
Shigella paradysenteriae (Boyd 1, 2, 3, Flexner 1, 2, 3, 4, 5, 6)	6, 52, 110, 360, 645	0.25 – 10.0
Shigella sonnei	6, 110	1.0 – 7.0
Family: Parvobacteriaceae		
Bacteroides fragilis	589	Insusceptible
Bacteroides fundiliformis	589	Insusceptible
Bacteroides sp	647	Insusceptible
Brucella abortus	52, 97, 646, 1147	0.5 – 3.75
Brucella melitensis	52, 360, 1147	0.5 –128.0
Brucella suis	52, 73, 1147	0 5 – 2.5
Donovania granulomatis	927	Sensitive‡
Hemophilus ducreyi	150, 151	1.0 – 15.0
Hemophilus hemolyticus	913	0 8 – 3 0
Hemophilus influenzae	52, 130, 150, 360, 495, 496, 525, 645	0 5 – 50 0
Hemophilus influenzae, a	130	2.5 – 5 0
Hemophilus influenzae, b	130, 166, 173	1.2 – 12.5
Hemophilus parainfluenzae	130, 913	2.5
Hemophilus pertussis	52, 64, 150, 274	1.2 – 50.0
Malleomyces mallei	52, C	10.00 –>10.0
Malleomyces pseudomallei	D, E	125.0 –>125 0

‡ Tested in chick embryo yolk sac culture.

TABLE 7—*Continued*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Family: Parvobacteriaceae (cont'd.)		
Pasteurella avicida	360, 1033	1.0 – 2.0
Pasteurella aviseptica	52, 360	2.0 – 15.0
Pasteurella boviseptica	360	2.0
Pasteurella equiseptica	360	1.0
Pasteurella hemolytica	1147	0.5 – 2 5
Pasteurella leptiseptica	6, 52	0.5 – 2 5
Pasteurella multocida	1147	1.0 – 10.0
Pasteurella pestis	77, 360, 447	0.5 – 6.0
Pasteurella suilla	360	2.0
Pasteurella tularensis	52, 103, 415	0.15 – 0.4
Streptobacillus moniliformis (*Haverhillia multiformis*)	947	Sensitive§
Family: Bacteriaceae		
Bacterium phosphorescens indigenus (*Photobacterium fischeri*)	656	200.0
Family: Bacillaceae		
Bacillus anthracis	52, 360, 1147	0.25 – 10.0
Bacillus cereus	52, 360	0.83 – 2.0
Bacillus megatherium	52, 360	0.25 – 4.0
Bacillus mesentericus	52, 360	1.0 – 1.67
Bacillus mycoides	6, 360, 656	0.13 – 8 0
Bacillus novus	360	0.5
Bacillus subtilis	6, 52, 138, 360, 656	0.056–128.0
Clostridium bifermentans (*Clostridium sordelli*)	6	Insusceptible
Clostridium botulinum	360	Insusceptible
Clostridium butyricum	360	Insusceptible
Clostridium histolyticum	360	Insusceptible
Clostridium lentoputrescens (*Clostridium putrificum*)	360	Insusceptible
Clostridium novyi	360	Insusceptible
Clostridium perfringens (*Clostridium welchii*)	6, 360	Insusceptible
Clostridium septicum	6, 360	Insusceptible
Clostridium sporogenes	360	Insusceptible
Clostridium tetani	6, 360	Insusceptible
Order. Actinomycetales		
Family. Mycobacteriaceae		
Mycobacterium avium	4, 502, 615	0.39 – 50 0
Mycobacterium lacticola (*Mycobacterium smegmatis*)	485, 656	1.0 – 1.38
Mycobacterium phlei	4, 52, 656	0.12 – 0.25
Mycobacterium ranae	338	0.5 – 1.0

§ Units per milliliter not stated.

TABLE 7—*Continued*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Order: Actinomycetales (cont'd)		
Family. Mycobacteriaceae (cont'd)		
Mycobacterium tuberculosis, var. *bovis*	502, 503, 645	0.095- 3.12
Mycobacterium tuberculosis, var. *bovis* (B.C.G.)	486	2.0 - 10 0
Mycobacterium tuberculosis, var. *hominis*	4, 338, 502, 503, 645, 738, 939	0.095- 12 5
Family: Actinomycetaceae		
Actinomyces bovis	52	4.0
Nocardia asteroides	4	4.0 - 12.5
Nocardia gypsoides	219	>1,000 0
Family: Streptomycetaceae		
Streptomyces albus	4	0.4 - 12.5
Streptomyces antibioticus	4	<0.4
Streptomyces griseus	4	>12.5
Streptomyces lavendulae	4	1.25
Order: Spirochaetales		
Family: Treponemataceae		
Leptospira andaman, a	555	<5.0
Leptospira australis, a	555	>50.0
Leptospira australis, b	555	<5.0
Leptospira autumnalis	555	<5.0
Leptospira bataviae	555	<5.0
Leptospira benjamin	555	<5.0
Leptospira canicola	555	<5.0
Leptospira djasiman	555	<5.0
Leptospira grippotyphosa	555	<5.0
Leptospira hebdomadis	555	<5.0
Leptospira icterohaemorrhagiae	555	<5.0 ->50.0
Leptospira javanica	555	<5 0
Leptospira naam	555	<5.0
Leptospira poi	555	<5.0
Leptospira pomona	555	<5.0
Leptospira pyrogenes	555	<5 0
Leptospira rachmat	555	<5.0 ->50 0
Leptospira samarang	555	>50 0
Leptospira sari	555	<5.0
Leptospira sejroe	555	<5.0
Leptospira sentot	555	<5.0
Leptospira species (Strains 90c, 3705, hc)	555	<5.0 ->50.0
Order: Rickettsiales		
Family: Rickettsiaceae‡		
Coxiella burnetii (*Rickettsia burneti*)	788	10 mg/egg

TABLE 7—*Continued*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Order: Rickettsiales		
Family: Rickettsiaceae‡ (cont'd.		
Rickettsia akari	483	10-20 mg/egg
Rickettsia orientalis (*Rickettsia tsutsugamushi*)	378	>2 mg/egg
Rickettsia orientalis (*Rickettsia tsutsugamushi*)	483	"Insusceptible"
Rickettsia prowazekii	378, 483	0.5-20 mg/egg
Rickettsia rickettsii	483	10-20 mg/egg
Rickettsia typhi (*Rickettsia mooseri*)	378, 483	2-20 mg/egg
Family: Chlamydozoaceae‡		
Miyagawanella felis (*Feline pneumonitis*)	610	Insusceptible
Miyagawanella lymphogranulomatis (*Lymphogranuloma venereum*)	252, 274, 610	Insusceptible
Miyagawanella ornithosis (*Meningo-pneumonitis*)	913	Insusceptible
Miyagawanella psittacii (*Psittacosis*)	252, 210, 274	Insusceptible
Order: Virales		
Family: Rabulaceae‡		
Rabula inflans (*Mumps Virus*)	1115	Insusceptible
Family: Charonaceae‡		
Tarpeia alpha (PR-8, Olson) (Human influenza A)	70, 175	Insusceptible
Tarpeia beta (Lee) (Human influenza B)	175	Insusceptible
Class: Eumycetes		
Alternaria sp	F	Insusceptible
Aspergillus fumigatus	28	Insusceptible
Aspergillus herbarorium	F	Insusceptible
Aspergillus niger	6	Insusceptible
Blastomyces brasiliensis	F	Insusceptible
Blastomyces dermatiditis	219, 249, F	Insusceptible
Candida albicans	219, F	Insusceptible
Candida candida	F	Insusceptible
Cladosporium sp.	F	Insusceptible
Coccidioides immitis	149, 219, F	Insusceptible
Cryptococcus neoformans	6, 28, 219, F	Insusceptible
Dematium sp	28	Insusceptible
Epidermophyton floccosum	219, F	Insusceptible
Epidermophyton inguinale	6	Insusceptible

TABLE 7—*Concluded*

MICROORGANISM	REFERENCES*	RANGE OF SENSITIVITY TO STREPTOMYCIN
		μg/ml
Class. Eumycetes (cont'd)		
Fusarium sp.	28, F	Insusceptible
Geotrichum sp.	219, F	Insusceptible
Histoplasma capsulatum	566, F	Insusceptible
Hormodendrum compactum	219, F	Insusceptible
Hormodendrum pedrosoi	219, F	Insusceptible
Microsporum audouini	F	Insusceptible
Microsporum canis	6, F	Insusceptible
Microsporum gypseum	F	Insusceptible
Monosporium apiospermum	219, F	Insusceptible
Mucor mucedo	F	Insusceptible
Neurospora sitophila	F	Insusceptible
Penicillium chrysogenum	6	Insusceptible
Penicillium expansum	F	Insusceptible
Penicillium luteum-purpurogenum	28	Insusceptible
Phialophora verrucosa	6, 219, F	Insusceptible
Rhizopus nigricans	F	Insusceptible
Rhodotorula sp	219	Insusceptible
Saccharomyces cerevisiae	219	Insusceptible
Sporotrichum schenkii	6, 219, F	Insusceptible
Trichophyton gypseum	6	Insusceptible
Trichophyton interdigitale	6	Insusceptible
Trichophyton mentagrophytes	28, F	Insusceptible
Trichophyton rubrum	219, F	Insusceptible
Trichophyton schoenleinii	F	Insusceptible
Trichophyton violaceum	F	Insusceptible
Phylum Protozoa		
Endamoeba histolyta	6, 638, 640, G	Insusceptible
Trichomonas fetus	68	Insusceptible
Trichomonas vaginalis	450	Insusceptible
Trypanosoma brucei	412, 424	Insusceptible
Trypanosoma equiperdum	412, 424	Insusceptible
Trypanosoma hippicum	412, 424	Insusceptible

Key to alphabetical references

A Reimann, H A , Chang, G C. T , Chu, L. W., Liu, P. Y. and Ou, Y. 1946 Asiatic cholera clinical study and experimental therapy with streptomycin. Amer Jour Trop Med , 26 631

B Loewe, L , Rosenblatt, P and Alture-Weber, E. 1946 A refractory case of subacute bacterial endocarditis due to *Veillonella gazogenes* clinically arrested by a combination of penicillin, sodium para-aminohippurate, and heparin. Amer. Heart Jour , 32 327

C. Miller, W. R , Pannell, L and Ingalls, M. S. 1948 Experimental therapy in glanders and melioidosis Amer. Jour. Hyg., 47 205.

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D. Gutner, L. B. and Fisher, M. W. 1948 Chronic melioidosis: discussion, case report, and special studies. Ann. Int. Med., 28: 1157.

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tion of *M. avium*, appear to be fairly uniform in their high degree of susceptibility to the bacteriostatic action of streptomycin. Only a few strains of organisms belonging to the *Actinomycetaceae* and *Streptomycetaceae* have been tested. Some of these are sensitive and some are resistant.

The only members of the order Spirochaetales tested *in vitro* are the Leptospira of the family *Treponemataceae*. Although there is considerable variation, most of these are relatively sensitive to streptomycin. Some *Borrelia* and *Treponema* have been found sensitive *in vivo*.

The reliability of some of the data given in table 7 is open to question. These tests for the sensitivity of microorganisms to streptomycin *in vitro* were performed in different laboratories, at different times, employing diverse cultural conditions and using streptomycin which probably varied in potency. The relative sensitivities to streptomycin of different strains of microorganisms, as tested in any one laboratory, are probably accurate. In some cases, however, a comparison of results obtained by different investigators using the same species brings to attention marked discrepancies.

The antibiotic action of streptomycin depends, in part, on the composition of the culture medium. For example, the presence of inorganic salts or glucose will reduce the bacteriostatic action, as will the addition of some substances, such as serum, which stimulate growth. Certain surface-active substances when added to the medium may increase the bacteriostatic action of streptomycin The pH of the medium is important, since the potency of streptomycin decreases as the hydrogen-ion concentration increases. Also, the activity of streptomycin is inversely proportional to the number of organisms employed in the test and is more effective on actively growing cells. These factors may be responsible for many of the discrepancies observed.

The greatest usefulness of streptomycin lies in its effectiveness for the therapy of certain infectious diseases. For this reason, in table 8 pathogenic microorganisms and their relative *in vitro* sensitivities to streptomy-

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Hormodendrum pedrosoi	219, F	Insusceptible
Microsporum audouini	F	Insusceptible
Microsporum canis	6, F	Insusceptible
Microsporum gypseum	F	Insusceptible
Monosporium apiospermum	219, F	Insusceptible
Mucor mucedo	F	Insusceptible
Neurospora sitophila	F	Insusceptible
Penicillium chrysogenum	6	Insusceptible
Penicillium expansum	F	Insusceptible
Penicillium luteum-purpurogenum	28	Insusceptible
Phialophora verrucosa	6, 219, F	Insusceptible
Rhizopus nigricans	F	Insusceptible
Rhodotorula sp.	219	Insusceptible
Saccharomyces cerevisiae	219	Insusceptible
Sporotrichum schenkii	6, 219, F	Insusceptible
Trichophyton gypseum	6	Insusceptible
Trichophyton interdigitale	6	Insusceptible
Trichophyton mentagrophytes	28, F	Insusceptible
Trichophyton rubrum	219, F	Insusceptible
Trichophyton schoenleinii	F	Insusceptible
Trichophyton violaceum	F	Insusceptible
Phylum· Protozoa		
Endamoeba histolyta	6, 63S, 640, G	Insusceptible
Trichomonas fetus	6S	Insusceptible
Trichomonas vaginalis	450	Insusceptible
Trypanosoma brucei	412, 424	Insusceptible
Trypanosoma equiperdum	412, 424	Insusceptible
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The greatest usefulness of streptomycin lies in its effectiveness for the therapy of certain infectious diseases. For this reason, in table 8 pathogenic microorganisms and their relative *in vitro* sensitivities to streptomy-

cin are listed. The division into sensitive, moderately sensitive, and resistant groups was made on the following basis:

Sensitive. Those microorganisms the growth of the majority of which is completely inhibited by less than 10.0 μg streptomycin per milliliter.

Moderately sensitive. Those microorganisms the growth of the majority of which is inhibited by between 10.0 and 100.0 μg streptomycin per milliliter.

Insensitive or resistant. Those requiring more than 100.0 μg of streptomycin per milliliter to inhibit.

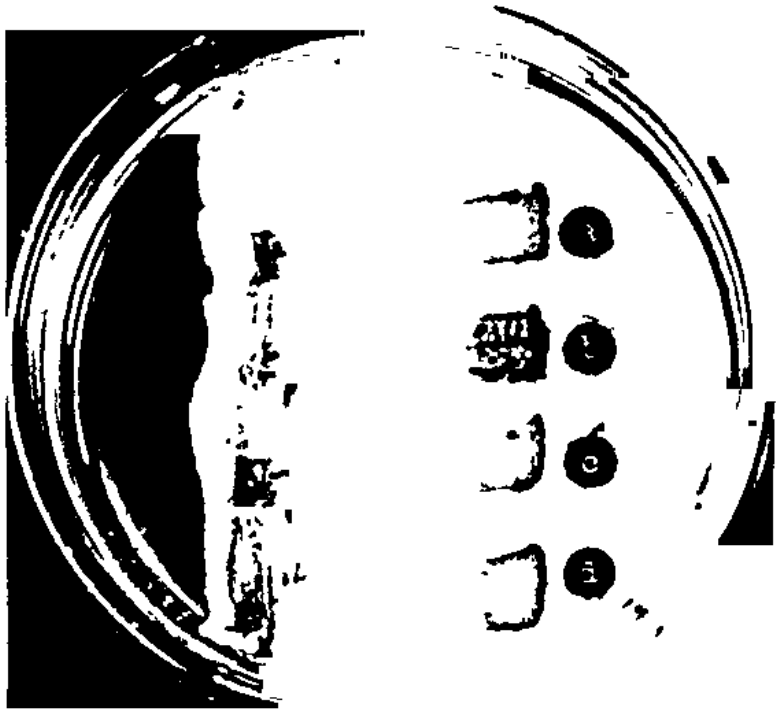


FIG 13 Inhibition of growth of sensitive bacteria by streptomycin-producing *S griseus* a = *E. coli*, b = *B mycoides;* c = *S. aureus;* d = *B subtilis* (Original).

Such a division is purely arbitrary, but is at least partly justified on the basis of the fact that marked differences in sensitivity of microorganisms to streptomycin *in vitro* do exist. Of more importance is the fact that clinical observations tend to confirm the validity of such a classification.

It is apparent from table 8 that a large number of pathogenic bacteria are highly susceptible *in vitro* to the antibiotic action of streptomycin. The most notable examples are the *Myobacterium, Pasteurella, Brucella, Hemophilus, Salmonella, Klebsiella,* and *Shigella* organisms. It cannot be assumed, however, that species of microorganisms highly susceptible *in vitro* to the antibiotic action of streptomycin will be equally susceptible *in vivo.* This is well illustrated by the relative ineffectiveness of streptomy-

TABLE 8

Sensitivity in vitro of pathogenic microorganisms to streptomycin

Sensitive

Actinomyces
Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Donovania granulomatis
Erysipelothrix rhusiopathiae
Hemophilus influenzae
Hemophilus pertussis
Klebsiella pneumoniae
Leptospira icterohaemorrhagiae
Listeria monocytogenes
(*Listerella monocytogenes*)
Mycobacterium tuberculosis, var. *hominis*
Mycobacterium tuberculosis, var. *bovis*
Neisseria gonorrhoeae
Neisseria meningitidis
(*Neisseria intracellularis*)
Pasteurella multocida
Pasteurella pestis
Pasteurella tularensis
Salmonella species
Salmonella typhosa
(*Eberthella typhosa*)
Shigella dysenteriae
Shigella paradysenteriae
Streptobacillus moniliformis
Veillonella gazogenes

Moderately sensitive

Aerobacter aerogenes
Alcaligenes faecalis
Corynebacterium diphtheriae
Coxiella burnetii
(*Rickettsia burneti*)
Diplococcus pneumoniae
Escherichia coli
Hemophilus ducreyi
Malleomyces mallei
Micrococcus pyogenes, var. *albus*
(*Staphylococcus albus*)
Micrococcus pyogenes, var. *aureus*
(*Staphylococcus aureus*)
Nocardia asteroides
Proteus morganii
Proteus vulgaris
Pseudomonas aeruginosa
(*Bacillus pyocyaneus*)
Rickettsia akari
Rickettsia prowazekii
Rickettsia typhi
(*Rickettsia mooseri*)
Streptococcus, alpha hemolytic
Streptococcus, beta hemolytic
Streptococcus faecalis
Vibrio cholerae
(*Vibrio comma*)

Insensitive

Bacteroides fragilis
Bacteroides fundiliformis
Clostridium species
Malleomyces pseudomallei
Rickettsia tsutsugamushi
(*Rickettsia orientalis*)
Virus of feline pneumonitis
Virus of human influenza
Virus of lymphogranuloma venereum
Virus of meningo-pneumonitis
Virus of mumps
Virus of psittacosis
Blastomyces dermatiditis
Candida albicans
Coccidioides immitis
Cryptococcus neoformans
(*Torula histolytica*)
Geotrichum species
Histoplasma capsulatum
Endamoeba histolytica
Trichomonas vaginalis
Trypanosoma species

cin therapy for the treatment of *Brucella*, *Salmonella*, and *Shigella* infections in humans. When a bacterium is susceptible to the action of strep-

tomycin *in vivo*, however, there is a correlation between the sensitivity *in vivo* and *in vitro*. Highly susceptible organisms respond to the action of streptomycin *in vivo* more readily than do those moderately sensitive. Those very resistant *in vitro* respond not at all *in vivo*.

Effect of Streptomycin on Morphology of Bacteria

Marked changes in the morphology of certain bacteria have been observed following exposure to streptomycin. These changes occur more frequently among the gram-negative bacilli than among the gram-positive (4).

Gram-negative bacilli, particularly *Shigella* and *Salmonella*, when exposed to slightly bacteriostatic concentrations of streptomycin, may become elongated and swollen and stain irregularly. Coccoid forms of *S. typhimurium* have been observed. Definite, but lesser degrees of change have been seen in *Aerobacter* and *Proteus*. No consistent changes were noted in *S. typhosus*, *Vibrio*, *E. coli*, and *Ps. aeruginosa* (4). *E. coli*, when exposed to streptomycin, has been reported to become either elongated or shortened and to grow in chains (5).

Cultures of *M. avium* and *M. tuberculosis* var. *hominis* have been reported to show an increase in granulation, a decrease in length, and a loss of acid-fastness after exposure to streptomycin (6).

Streptomycin also has been reported as having a lytic action on *B. subtilis* cells (1).

Dihydrostreptomycin and Mannosidostreptomycin

Dihydrostreptomycin has been reported as essentially equal to streptomycin in its bacteriostatic action for *E. coli*, *B. subtilis*, *S. marcescens*, *B. mycoides*, *S. schottmülleri*, *Sh. paradysenteriae* (Sonne), *Kl. pneumoniae*, *S. aureus*, Streptococcus, *M. tuberculosis* (H37Rv) (7).

On the other hand, although dihydrostreptomycin was reported to be approximately equal in activity to streptomycin against *A. aerogenes*, *B. subtilis* (Merck), *Kl. pneumoniae*, and *S. marcescens*, it was found to be less active against one strain each of *S. aureus*, *Ps. aeruginosa*, *B. subtilis*, *M. tuberculosis*, and *Bacillus* sp. strain 290 (8).

More recent studies indicate that dihydrostreptomycin and streptomycin have a closely comparable bacteriostatic action *in vitro* on tubercle bacilli (9, 10, 11).

The relative sensitivity *in vitro* of a variety of microorganisms to streptomycin, dihydrostreptomycin, mannosidostreptomycin, and dihydromannosidostreptomycin was brought out in table 3. Dihydrostreptomycin is less active than streptomycin against some of the organisms, especially *S. schottmülleri* and *S. typhosa*. The activity of dihydrostreptomycin for

tubercle bacilli, with the exception of one strain, "K," is essentially the same as that of streptomycin.

The bacteriostatic action of both mannosidostreptomycin and dihydromannosidostreptomycin is significantly less than that of streptomycin and dihydrostreptomycin for all the organisms, with the exception of *S. typhosa* and *S. schottmulleri*. These results are similar to those previously reported (12).

Miscellaneous Activities and Applications of Streptomycin in vitro

Although streptomycin is inactive against filterable viruses, it can inactivate *E. coli* and *S. aureus* bacteriophage strains (13).

Streptomycin had no effect on tetanus toxin after 48 hours' exposure (14).

The fact that streptomycin in high concentrations can be bacteriostatic or bactericidal for many types of bacteria is applied where selective culture media are desired. The antibiotic can be added to agar media for isolation of pathogenic fungi (15); it can be used to render cultures of protozoa bacteria-free (16, 17); and, in virus studies, it can be used to protect chick embryos from bacterial contaminants (18, 19).

Streptomycin has been used in the preservation of milk (20) and of bull semen (21). Limited study has indicated that it may have some usefulness for elimination of various plant pathogens (22).

Methods of Testing Streptomycin Sensitivity

Since bacteria vary in their sensitivity to streptomycin and are also capable of becoming resistant to the antibiotic, the testing of sensitivity to streptomycin becomes an important laboratory determination. An *in vitro* test is performed with the object of finding the least amount of streptomycin, expressed in micrograms or units per milliliter, that will effect complete bacteriostasis. The direct result of this test will provide a quantitative estimate of the action of streptomycin and a characterization of the organism in question as being sensitive or insensitive to streptomycin *in vitro*.

The procedure for the determination of streptomycin sensitivity is essentially one of exposing a culture of actively growing bacteria to a graded series of streptomycin concentrations in an appropriate culture medium and, after a sufficient period of incubation, recording the least concentration of streptomycin that caused complete inhibition of growth. It is of considerable importance to observe these general precautions:

1. The culture medium should be sufficiently nutritive to permit adequate growth.

2. Substances which may materially interfere with or potentiate the action of streptomycin, directly or indirectly, should not be present.

3. The pH of the culture medium should be neutral or slightly alkaline.

4. The temperature of incubation should be optimal for the test organism.

5. The inoculum of test organisms should be large enough to include a representative portion of the growth, since there may be variation in sensitivity to streptomycin among bacteria of a single strain. The inoculum should not be so large, however, that there is an appreciable dilution of streptomycin in the test medium or that the true sensitivity of the organism may be obscured.

6 The streptomycin used should be of known potency.

7. Control media, without streptomycin, should be inoculated and run in parallel with the test.

Many procedures have been devised for determining sensitivity to streptomycin, and a few that have found wide application are described below:

Indirect methods

The test organism is subcultured after original isolation and used as a pure culture.

LIQUID MEDIUM

Dilutions of streptomycin are made up in sterile broth. These may be in twofold increments, as for example: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 μg/ml of the medium. Each tube is inoculated with the test organism and incubated, usually at 37° C for 18 to 24 hours. Mycobacteria will require a much longer period of incubation.

This method is especially suitable for laboratories that perform streptomycin-sensitivity tests infrequently.

SOLID MEDIUM

Agar media are prepared with varying concentrations of streptomycin as previously described for liquid media. Since streptomycin is to some extent thermolabile, it must be added aseptically to the sterilized and cooled agar base. The media are distributed in sterile petri dishes, and a number of different strains of bacteria may be inoculated on the same plate. A single loopful of broth culture is streaked radially on the plate. In this manner it is possible to test as many as sixteen strains on a standard petri dish.

This procedure is recommended for laboratories that perform many sensitivity tests. "Spreaders," such as *Proteus* species, should not be tested with other bacteria on the same plate (fig. 14).

Direct methods

Material from infectious processes may be inoculated directly on media containing streptomycin.

A solid medium should be used for this test. This procedure has two distinct advantages over the indirect methods of testing: (a) by elimina-

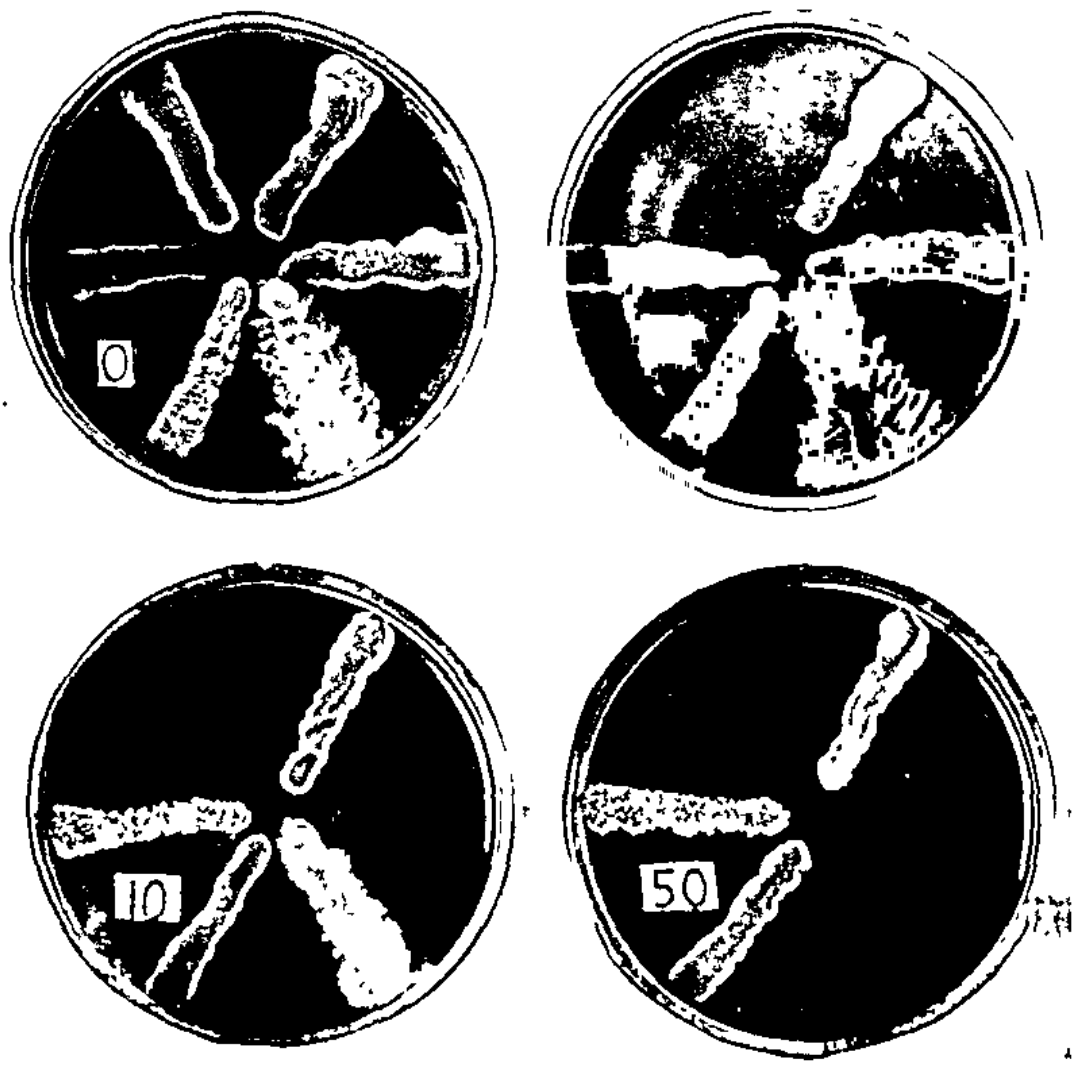
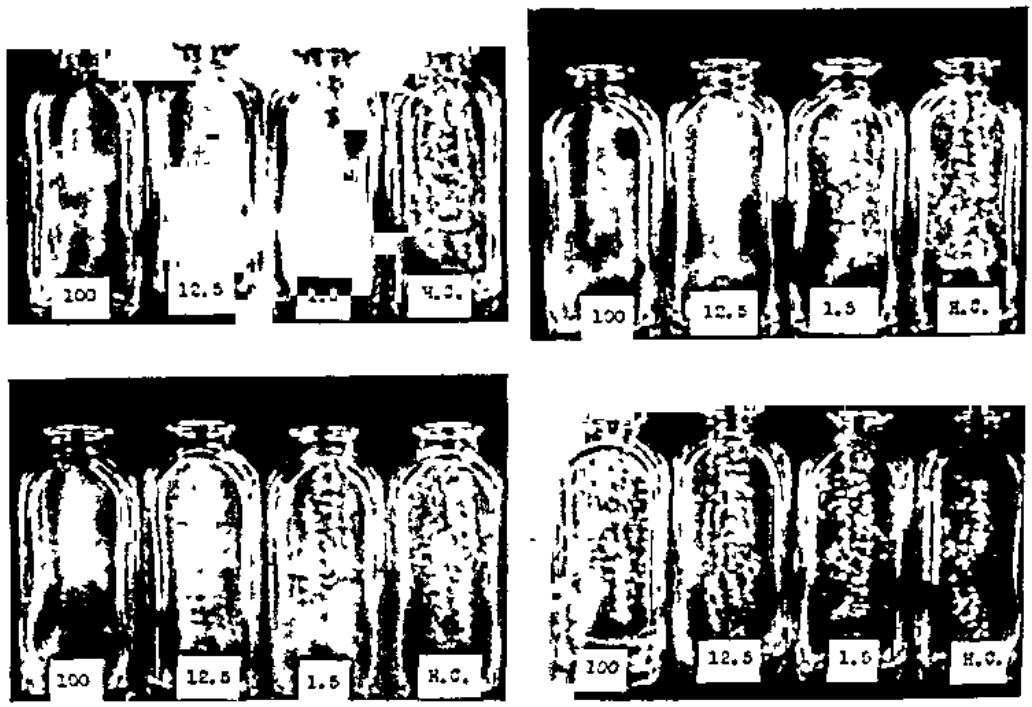


FIG. 14. Streak plate method for the determination of sensitivity of microorganisms to streptomycin, μg/ml of medium.

tion of subculturing, the time required for the test may be shortened, and (b) it will indicate the relative numbers of streptomycin-sensitive and -resistant organisms within a given specimen. The disadvantages are that irregular results will be obtained if bacteria are not evenly distributed throughout the specimen, and, in specimens containing several types of bacteria, the growth of one organism may be obscured by the growth of another.

This method has found its greatest application in the determination of streptomycin sensitivity of tubercle bacilli, often shortening by several weeks the time involved. The specimen to be tested, such as sputum, is first digested to homogenize the specimen and to eliminate contaminating organisms. It is then concentrated, and the material is inoculated on tubes of egg-yolk agar previously prepared to contain varying concentrations of streptomycin. If large numbers of tubercle bacilli are present in the original specimen, results may be evident within 2 weeks of incubation (23) (fig. 15).



A standard reference strain of bacteria may be run in parallel with other organisms, to be used as a basis for the calculation of end points. This is done where the potency of streptomycin preparations may vary from test to test and when it is desired to check on the test medium (24).

Miscellaneous methods

1. An indicator may be used in the test medium as a means of revealing the presence of growth through a color change. For coliform and related bacteria, glucose and a pH indicator may be added to the medium (25). An objection to the latter procedure is that a shift in pH toward acidity will correspondingly decrease the activity of streptomycin (fig. 16).

2. The bacteria to be tested are streaked evenly on an agar medium in a petri dish, and a filter disc saturated with stréptomycin is placed on the surface of the inoculated medium (26). A number of discs, with varying concentrations of streptomycin, may be used on one or a series of plates. After incubation, a zone of inhibition, roughly proportional in size to the sensitivity of the organism, will appear around the disc. A pure culture of bacteria may be used, or the specimen may be inoculated directly on the

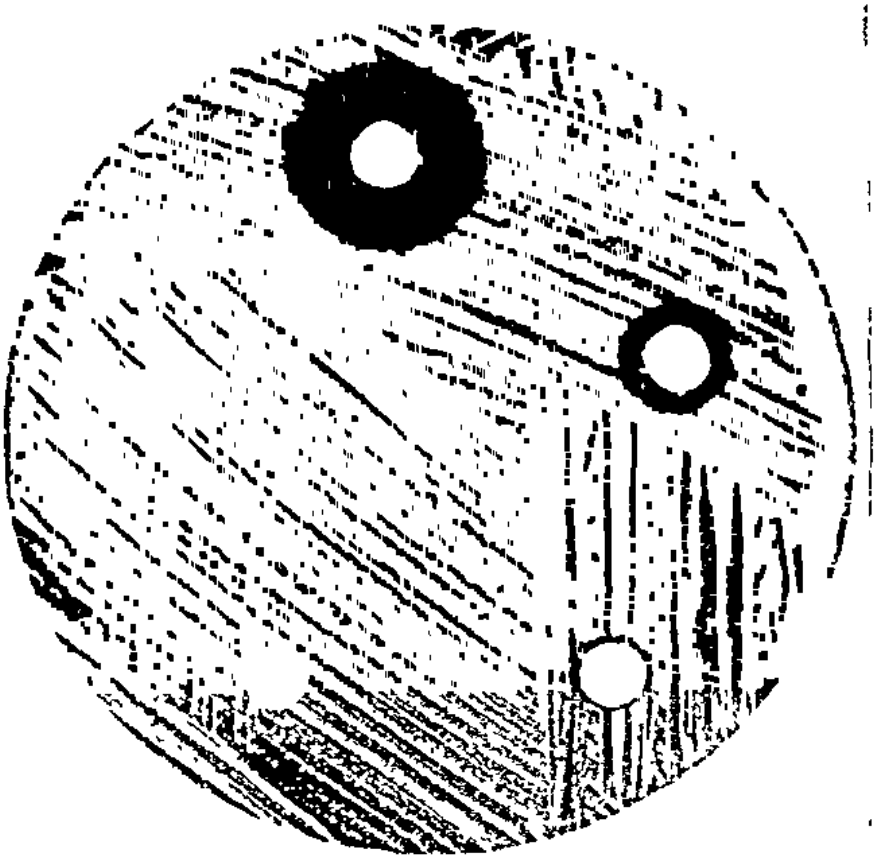


FIG. 16. Filter paper disc method for the determination of sensitivity to streptomycin.

plate. This procedure is essentially qualitative, for a number of factors other than the sensitivity of the organism will affect the size of the zone.

3. A correlated *in vitro-in vivo* test may be made by using one of the previously described *in vitro* methods and infecting a chick embryo or susceptible laboratory animal with the microorganism to be tested (27, 28). By treating the infected egg or animal with streptomycin and later assaying the therapeutic effect, one can obtain an *in vivo* sensitivity determination.

Since streptomycin will retain its potency for a considerable time at low temperatures, streptomycin test media may be prepared in advance

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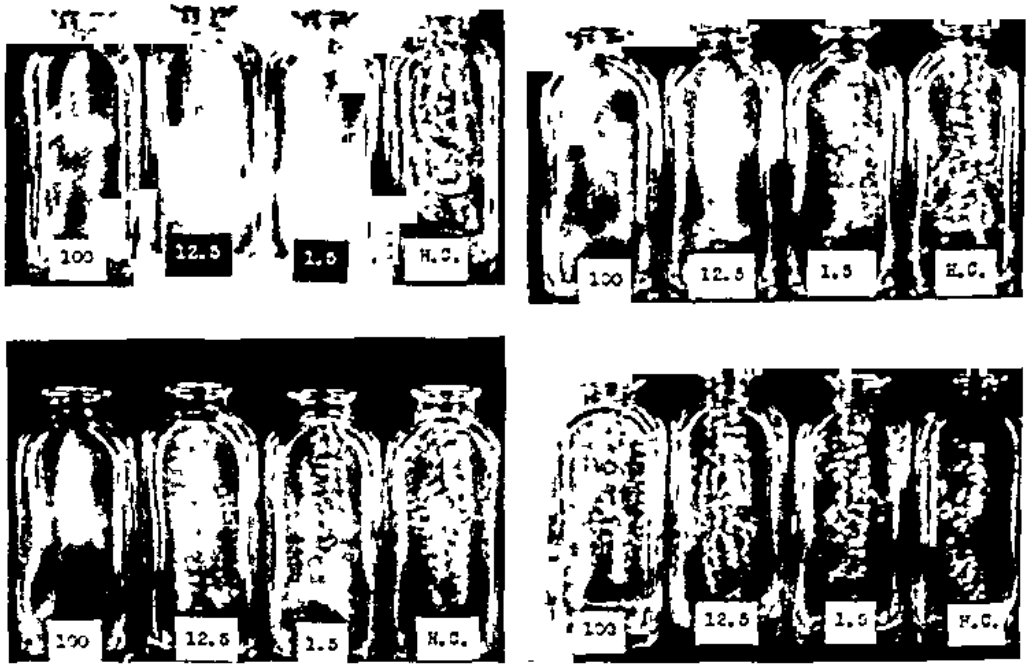

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In recording results from either solid or liquid media, there should be a definite endpoint, with a particular concentration of streptomycin causing complete inhibition. The least amount of streptomycin used that prevents growth of the test organism should be reported. This may involve use of the terms *sensitive* or *resistant*, which may in some cases lead to confusion. As an example, an organism is tested which grows in the presence of 6.25 μg of streptomycin per milliliter, but is inhibited by 12.5 μg/ml. This may be reported as: 1. "Organism sensitive to 12.5 μg streptomycin per milliliter," or 2. "Organism resistant to 6.25 μg/ml; sensitive to 12.5 μg/ml."

A strain of bacteria, though in pure culture, may consist of organisms having different sensitivities to streptomycin, with the result that a sharp endpoint will not be obtained. In liquid or solid media there may be a "tapering-off" of growth through increasing concentrations of streptomycin. As an example, when the twofold dilutions previously mentioned are used, the test organism may show 4+ growth in 1.56 μg of streptomycin per milliliter of medium, 3+ growth in 3.13, 2+ growth in 6.25, 1+ growth in 12 5, a "trace" in 25, and complete inhibition in 50. In this case, the test may be reported as: "Partial inhibition from 3.13 through 25 μg streptomycin per milliliter, almost complete by 12.5 μg/ml."

When a solid medium is used, it may be possible to report the number of colonies occurring in higher concentrations of streptomycin, as well as the approximate relative numbers of streptomycin-sensitive and streptomycin-resistant organisms.

It would be highly desirable for laboratories to use a standard test. In many instances the decision of whether a patient will receive streptomycin will be based on the results of a sensitivity determination, and if the test is performed without due regard for factors that may seriously affect the activity of streptomycin, a false impression may be obtained. Since it is unlikely that a universal procedure will be adopted, the alternative is fundamentally one of being consistent A procedure used by a laboratory should be rigidly controlled, and a standard reference strain should be used at intervals as a check on the performance.

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tion of dihydrostreptomycin, organisms resistant to other drugs, that is, sulfonamides, sulfones, or penicillin, are not necessarily resistant to streptomycin *in vivo* or *in vitro*. The antibiotic can therefore be used with effect in some infections, due to organisms of relatively low sensitivity to the drug, in which streptomycin would not be the drug of choice, but in which organisms resistant to the better drugs are involved. Organisms resistant to dihydrostreptomycin are resistant, however, to streptomycin and *vice versa*.

Pasteurella Infections

Studies by a number of workers attest to the efficacy of streptomycin in the treatment of experimental infections with *P. pestis*. Among the experiments of Wayson and McMahon (1), Hornbrook (2), Herbert (3),

TABLE 9

*Effect of streptomycin treatment on mice infected subcutaneously with P. pestis** (3)

STREPTOMYCIN TREATMENT STARTED	SURVIVORS (21 DAYS)	MEAN DEATH TIME
		days
Immediately after infection	17/20 (85%)	6.8
24 hours after infection	12/20 (60%)	8 1
48 hours after infection	1/20 (5%)	5 8
72 hours after infection	0/20	6.0
Controls (no treatment)	0/20	3.8

* Mice 16–18 gm. Infecting dose 349 viable organisms subcutaneously (in back). Streptomycin doses of 400 units in 0.2 ml saline solution injected intraperitoneally twice daily for 2½ days (5 doses = 2,000 units in all)

Quan, Foster, Larson, and Meyer (4), Meyer, Quan, and Larson (5), and Sokhey and Wagle (6) are examples of the protection of mice or guinea pigs infected by injection by various routes, by inhalation, and by the bites of infectious fleas.

Quan *et al.* (4) found that mice, treatment of which was begun 48 hours after subcutaneous inoculation, at which time the infection was well advanced, remained well after administration of 500 μg of streptomycin every 3 hours for 3 days, a total of 12 mg. When one intraperitoneal treatment was given on the 48th hour after infection, the median effective dose was 1,000 to 1,250 μg for a mouse weighing 20 gm. When mice were infected intranasally with *P. pestis* and treatment was begun 36 hours later with 200 to 400 μg of streptomycin administered intraperitoneally every 6 hours for 10 days, 90 to 95 per cent survived. Treatment with a combination of sulfadiazine and antiplague serum was less effective than treatment with

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CHAPTER 8

ACTIVITY OF STREPTOMYCIN IN EXPERIMENTAL INFECTIONS

The activity of streptomycin in experimental infections *in vivo* will, of course, depend, as it does in man, on the concentrations achieved actually at the locus of infection, as well as on the susceptibility of the given organism to streptomycin under the given conditions. Such concentrations will be a function not only of the dosage regimen, that is, the pharmaceutic form of the drug, the route of administration, and the frequency of dosage, but also of conditions in the host. The latter factor will, of course, be most important in the case of subacute or chronic infections in which tissue reactions may produce alterations, in supply of blood and tissue fluids to the locus, of greater or lesser extent.

For the purposes of the present discussion, it is presumed that all these factors have been recognized and that the results obtained are those to be expected when such factors have been so arranged as to be optimal for the host in resistance to the parasite. When, in any given work, such is clearly not the case, attention will be drawn to that fact.

Most of the known data bearing on the problem of achieving optimal and active concentrations of the antibiotic at the locus of infection are discussed elsewhere in the present volume. Thus, the effect of changes in the surrounding menstruum on the antibacterial activity of the antibiotic are discussed in the chapters on antibacterial activity of streptomycin *in vitro* (chapter 7) and mode of action of streptomycin (chapter 12). What is known concerning the factors influencing concentration of the antibiotic at any given place or time is fully covered in the chapter on the absorption, distribution, and excretion of streptomycin (chapter 14).

One other general statement on the activity of streptomycin can be made. Presumably because its mode of action is different from that of all other presently employed chemotherapeutic substances, with the excep-

Brucella Infections

In an early experiment Jones *et al.* (14) infected chick embryos with *Br. abortus* and treated them with crude streptomycin. The results of cultures of the embryos indicated that treatment with approximately 300 to 600 units of the drug eliminated the organism.

After this, studies of the effect of streptomycin on mice or guinea pigs infected with *Br. abortus* or *Br. suis* were reported by Live, Sperling, and Stubbs (15), by Gilman and LeGrow (16), and by Kelly and Henley (17). In general, their results indicated that streptomycin exercised some suppressive effect on the infections as evidenced by cultures and observations of the liver, spleen, and lymph nodes of the animals. Only by prolonged treatment with large doses of the drug was the organism eliminated from the animals and then with no regularity. In one experiment Live, Sperling, and Stubbs (15) inoculated guinea pigs with 1,000 infective doses of *Br. abortus* by subcutaneous injection and treated them for 24 days with 20,000 units of streptomycin daily divided into six equal doses. When examined 1 to 15 days after cessation of treatment, only seven of thirty-five animals yielded positive cultures.

Finally, Hall and Spink (18) and Shaffer and Spink (19, 20) studied the effect of streptomycin and other agents on chick embryos infected with *Brucella* via the allantoic sac or the yolk sac. They concluded that streptomycin exercised a protective effect but that its combination with sulfadiazine was more effective than either drug alone.

Hemophilus Infections

That *H. influenzae* infections in mice are very susceptible to treatment with streptomycin is indicated by the work of Hewitt and Pittman (21) and Alexander and Leidy (22). The former workers infected mice intraperitoneally with approximately 100,000 M.L.D. of a sensitive strain of the organism suspended in mucin and found that the smallest total amount of streptomycin giving 50 per cent protection was 160 units. The drug was administered subcutaneously in three equal doses, the first dose being given 30 minutes before inoculation and two subsequent doses being given at 4-hour intervals. Streptomycin was the most effective single agent of the several agents that were tested against such an infection.

Alexander and Leidy (22) determined the smallest single intraperitoneal dose of streptomycin which would protect 50 per cent of mice against 100,000 to 1,000,000 LD_{50} of *H. influenzae*. With the several sensitive strains of the organism studied, the minimal effective dose varied between 19 and 39 units.

Mice infected with *H. pertussis* are partly protected by treatment with streptomycin. In one experiment by Hegarty, Thiele, and Verwey (23)

streptomycin. When guinea pigs were infected subcutaneously with 1,000 M.L.D. of organisms, treatment beginning 120 hours after infection with 10 mg per day for 10 days protected 80 to 100 per cent of the animals.

One of the experiments by Herbert (3) is summarized in table 9.

Several reports indicate that streptomycin is effective in the treatment of *P. tularensis* infections in mice. In a study by Heilman (7), mice were infected intraabdominally with 10 to 100 lethal doses of the organism and treatment was begun 7 to 8 hours after infection. The drug was administered subcutaneously at intervals of 3 to 4 hours, five times during the day for 10 days. Mice treated with 1,000 units per day all survived. Of mice receiving 500 units per day, 42 per cent survived. The results of the experiment are summarized in table 10.

TABLE 10

Effect of streptomycin on deaths of mice inoculated with P. tularensis (7)

Treatment	MICE THAT DIED EACH DAY AFTER INOCULATION										NUMBER OF MICE THAT DIED	MORTALITY RATE
	Days after inoculation											
	1	2	3	4	5	6	7	8	9	10 to 13		
												per cent
Treated with 1,000 units per day (30 mice)	0	0	0	0	0	0	0	0	0	0	0	0
Treated with 500 units per day (12 mice)	0	0	0	0	0	1	1	2	0	1	5*	42
Untreated (30 mice)	0	0	13	17	0	0	0	0	0	0	30	100

* Two other mice died of pneumonia on the 26th and 32nd days respectively.

Chapman, Coriell, Kowal, Nelson, and Downs (8) infected mice intradermally with 15 to 20 M.L.D. of *P. tularensis*. When therapy was delayed for 24 to 72 hours after infection, 10,000 units of streptomycin per kilogram given subcutaneously every 3 hours for 10 days resulted in survival rates of 80 to 100 per cent of the animals. Likewise, Tamura and Suyemoto (9) found the drug effective in experimental tularemia infections in mice.

With turkeys inoculated intravenously with *P. multocida*, McNeil and Hinshaw (10, 11) found that death was prevented by intramuscular administration of 150,000 units of streptomycin within 6 hours after infection.

When mice were infected intranasally or intracerebrally by Jawetz (12, 13) with an unknown species of *Pasteurella*, the administration of relatively large doses of streptomycin resulted in partial protection.

E. coli

Leopold and associates (34) infected the eyes of rabbits with *E. coli* by injecting 20,000 organisms into the vitreous humor. The infection was controlled when 100,000 μg of streptomycin was injected into the vitreous within 30 minutes after inoculation. Administration of the drug by retrobulbar injection plus iontophoresis or by anterior chamber injection resulted in partial protection.

TABLE 11

Effect of treatment with streptomycin on experimental infections in mice with microorganisms of the Friedlander group

EXPERIMENT	INFECTING DOSE			TREATMENT WITH STREPTOMYCIN*		MICE INOCULATED	MICE THAT DIED	MORTALITY
	Strain	Number of times lethal dose	How given	Units per day	Number of days			
								per cent
1	No. 575 Type unknown	10,000	Intra-abdominally	185	3	8	0	0
				460	2	6	0	0
				None		14	14	100
2	No. 837 Type A	1,000	Intra-abdominally	500	3	20	1	5
				250	3	20	6	30
				None		20	20	100
3	No. 838 Type A	1,000	Intra-abdominally	500	3	15	0	0
				None		15	15	100
4	No. 837 Type A	100	Intra-nasally	500	3	10	8	80
				None		10	10	100
5	No 837 Type A	100	Intra-nasally	500	7	15	2	13
				None		15	15	100

* Treatment was started 3 hours after infection In experiment 1 the mice were treated five times a day. In the remaining experiments treatment was administered twice a day.

Salmonella Infections

That mice infected with lethal doses of *S. schottmulleri* could be protected by treatment with streptomycin was shown early by Jones *et al.* (14) and by Robinson, Smith, and Graessle (35). Using a similar type of infection, Rake and his colleagues (36) found both streptomycin and streptomycin B to be effective in a ratio of 1 to 2.7 in terms of weight.

Slanetz (37) incorporated streptomycin in the drinking water to eliminate *S. enteritidis* from a colony of mice used for the production of enceph-

mice inoculated intracerebrally with 5,000 organisms were given streptomycin intraperitoneally every 6 hours for 10 days, starting immediately after infection. Treatment with 2 mg of the drug per day resulted in 50 per cent survival. Likewise, Bradford and Day (24) found that, with mice inoculated intranasally with one strain of the organism and treated subcutaneously with 130 units of the drug four times daily for 4.5 days, more than ten times the number of organisms were required to kill 50 per cent of the treated mice than the untreated.

Rabbits were inoculated intradermally with *H. ducreyi* by Mortara and Saito (25) and treated with streptomycin given intramuscularly. The development of lesions was prevented by 150,000 units of streptomycin divided into three equal doses given within 24 hours, when treatment was started within 4 hours after inoculation.

Klebsiella Infections

Several reports indicate that streptomycin is effective in protecting mice infected with *Kl. pneumoniae*. Table 11 illustrates the results of experiments by Heilman (26) in which the drug was administered subcutaneously. When the mice were infected by the intranasal route, longer treatment was necessary for survival.

Kolmer (27) and Zubrod (28) likewise obtained protection. Hadley, Laurent, and Onslow (29) found streptomycin highly effective in preventing fatal pneumonia in rats after the intrabronchial inoculation of Friedlander organisms when the drug was administered intramuscularly or inhaled as an aerosol.

D. granulomatis

D. granulomatis, recently isolated from cases of granuloma inguinale and believed to be the etiologic agent (30), has proved to be a gram-negative bacterium which more recent evidence has tended to relate to the genus *Klebsiella* in the tribe Escherichiae (31). As might be expected, were such a relationship actually to be proved, *D granulomatis* has been shown to be highly sensitive to streptomycin. Thus Dunham and Rake (32) showed that, *in vivo*, in the embryonated egg, and *in vitro*, streptomycin, with streptothricin, was the most active chemotherapeutic compound of natural or synthetic origin tested. This report was soon followed by one of clinical success in the treatment of twenty-three patients (33). Donovan bodies disappeared from the lesions in 5 to 9 days. In subsequent laboratory studies Rake and Dunham (32A) expanded the earlier studies and showed that streptomycin had an actual bactericidal activity. In a combined *in vitro* and *in vivo* test streptomycin was active at 4 units/ml when tested in the embryonated chicken egg.

Using chick embryos infected on the chorioallantois, Ordal and Meyer (44) obtained 50 per cent protection by treatment, 6 hours after inoculation, with three doses containing a total of 150 units of the drug.

Pseudomonas

Jones *et al.* (14) succeeded in protecting mice injected with lethal doses of *Ps. aeruginosa* by the administration of four doses of streptomycin at 6-hour intervals, the total amount of drug per mouse being 190 units. Under the conditions of his experiments Robinson (43) failed to attain protection of animals with a similar type of infection.

The abraided corneas of rabbits were infected with *Ps. aeruginosa* by Bellows, Burkholder, and Farmer (45, 46) and treated with streptomycin. Three instillations, at 2-hour intervals beginning 6 hours after inoculation, of a solution containing 10,000 mg of the drug per milliter afforded complete protection.

Malleomyces

Miller, Pannell, and Ingalls (47) studied the chemotherapy of experimental infections with *M. mallei* and *M. pseudomallei* in hamsters. They concluded that streptomycin had little effect on established infections with either organism but found sulfadiazine to be very effective.

Neisseria

The meningococcus is susceptible to streptomycin *in vivo*. Miller and Bohnhoff (48) infected mice by intraperitoneal inoculation of approximately 100,000 lethal doses of *N. meningitidis* suspended in mucin. With normally susceptible strains of the organism, complete protection was attained with two subcutaneous doses of streptomycin, each containing 50 units, administered 3 and 6 hours after infection.

S. moniliformis

Streptomycin was found superior to penicillin by Levey and Levey (49) for treatment of mice with involvement of joints following intravenous infection with *S. moniliformis*. Administration of 5,000 units of streptomycin three times a day for 3 days cured 85 per cent of the animals. When doses were halved, 76 per cent were cured.

Pleuropneumonia-like Organisms

Rats were infected intravenously with pleuropneumonia-like organisms and treated with streptomycin by Powell, Jamieson, and Rice (50, 51). When treatment was started 1 hour after infection, death or the development of disabling arthritis was prevented by the administration of 1,000

alitis vaccine. The animals were treated for 7 days and received approximately 100 units a day.

Streptomycin was found by Benson (38) to be effective in protecting 2-day chicks inoculated with *S. pullorum*. When five injections, each containing 2,500 units of the drug, were given at 12-hour intervals starting 16 hours after infection, 82 per cent survived. Of the untreated controls 19 per cent survived.

In mice infected with *S. typhosa*, Welch *et al.* (39) found the mortality rate to be increased by small doses of streptomycin, whereas larger doses afforded protection. Hobby and Lenert (40) obtained increased survival rates in mice infected intraperitoneally with *S. typhosa* suspended in mucin and treated with three doses of streptomycin or with a fraction remaining after the removal of streptomycin from crude material.

Rats that had ulcerative cecitis were given 0.1 gm daily of streptomycin in the drinking water by Bloomfield and Lew (41). Treatment for 10 days resulted in a striking therapeutic effect. Although the primary cause of the disease is not known, the organisms of the *Salmonella* group constantly associated with the disease disappeared after the treatment.

Rake, Pansy, Jambor, and Donovick (40A) demonstrated that, although 3.6 times as much dihydrostreptomycin as streptomycin was required to inactivate *S. schottmülleri in vitro*, only 1.9 times as much was required in standardized infections *in vivo* with this organism in mice. Rake and Donovick (40B) examined the effect of single as compared to divided dose schedules in the same standardized infection. Using crystalline streptomycin, they showed that the CD_{50} for a single dose in different experiments lay between 3.0 and 5.9 mg/kg, whereas when the same amount was divided into three injections a day the CD_{50} lay between 7.5 and 11.5 mg/kg. Thus when divided doses were used, more than twice as much streptomycin was required to protect 50 per cent of the mice.

Shigella

The effect of treatment with streptomycin on *Sh. gallinarum* infections in the chick embryo was studied by Jones, Metzger, Schatz, and Waksman (14). Although many of the embryos died from causes other than the infecting organism, the results of cultures of the embryos indicated that 150 to 300 units of streptomycin protected against the infection.

Proteus Infections

The workers at Rutgers University (14, 42) reported that animals infected with *Pr. vulgaris* and also with a mixed culture of this organism and an anaerobic streptococcus were protected by administration of streptomycin. Robinson (43) was less successful.

Listeria

Gray, Stafseth, and Thorp (56) examined the effect of streptomycin on the blood and temperature of rabbits infected with *L. monocytogenes* and reported, "The data obtained would suggest that a large dose of streptomycin given during the early stage of the disease may be of some value."

B. anthracis

Streptomycin was found by Miller, Scott, Noe, Madin, and Henley (57) to be highly effective and superior to penicillin and sulfadiazine in the treatment of *B. anthracis* infections in mice. The mice were infected subcutaneously with at least 100 M.L.D. of *B. anthracis* spores and treated by administration of streptomycin subcutaneously every 3 hours for 7 days. In one experiment in which treatment was started 24 hours after infection, doses of 100 to 200 units of streptomycin, 800 to 1,600 units a day, resulted in survival of 85 to 92 per cent of the animals.

Employing a strain of *B. anthracis* only slightly susceptible to streptomycin *in vitro*, Kolmer (27) obtained somewhat less favorable results with the drug.

Clostridium Infections

Streptomycin has not been found effective in experimental *Clostridium* infections. Ryan *et al.* (58) infected mice with the various clostridia which cause gas gangrene. A single topical application of the drug to the infected, wounded thighs of mice was without effect except that it prolonged the life of mice infected with *C. perfringens* 52 per cent. In experimental *C. perfringens* infections in guinea pigs, according to Robinson (43), streptomycin had no influence on the disease, even when the drug was injected at the site of the infection.

Agrobacterium

Crown gall, a bacterial infection of plants, was treated by Hampton (59) with streptomycin. Local application of the drug resulted in cure of the disease in several different genera.

Spirochetal infections

In experimental infections with *B. novyi* and *L. icterohaemorrhagiae*, streptomycin was found by Heilman (60, 61) to be active against both organisms, although less so than penicillin. In the case of *B. novyi*, he used Swiss mice and showed 1,000 units a day in five divided doses produced some clearing of the blood and less frequent relapses than in the control group. Syrian hamsters were used for the work with *L. icterohaemorrhagiae*. In this case 1,000 units of streptomycin a day in four divided doses protected

units of streptomycin, given three times a day for 3 or 4 days. When treatment was delayed, larger doses of the drug were required.

M. PYOGENES

That experimental staphylococcal infections produced by normally sensitive strains of *M. pyogenes* can be readily controlled by streptomycin has been reported by Robinson, Smith, and Graessle (35) and by Wolinsky and Steenken (52). The latter workers protected mice infected intraperitoneally with lethal doses of the organism by intraperitoneal injection of 500 units of the drug at the time of inoculation and repeated once 1.5 hours later.

STREPTOCOCCUS

Large amounts of streptomycin were found by Robinson (43) to be necessary to protect mice infected with lethal doses of hemolytic streptococci. When *S. pyogenes* was inoculated into the vitreous humor of the eyes of rabbits, Bellows et al. (45, 46) were able to prevent development of an abscess by injecting as little of 25 μg of the drug into the vitreous within 6 to 8 hours after infection

Rake and Donovick (40A) have demonstrated that there is no significant difference between dihydrostreptomycin and streptomycin in the amount required to protect 50 per cent of the mice infected with 500 lethal doses of *S. pyogenes* C-203. It has also been possible to demonstrate (40B) that in standardized *Streptococcus* infections in mice, as in infections by *S. schottmülleri*, approximately twice as much pure streptomycin or dihydrostreptomycin is required with a divided dose schedule of three doses a day as with a single dose.

D. PNEUMONIAE

Mice were infected with many lethal doses of *D. pneumoniae* by Robinson, Smith, and Graessle (35) A single subcutaneous injection of 1,600 units of streptomycin immediately after infection resulted in survival of all of the treated animals.

ERYSIPELOTHRIX

Mice infected with *E rhusiopathiae* were treated with streptomycin by Klauder and Rule (53) Relatively large doses of the drug produced only a slight therapeutic effect Woodbine (54) obtained somewhat better results but estimated that penicillin was five times more effective on a weight-for-weight basis. Grey (55) found that a single dose of 140,000 μg of streptomycin administered to turkeys 24 hours after infection prevented death.

experiments were carried out in the developing chicken embryo, the streptomycin being administered immediately before the infective inoculum. Effective doses were from 20 mg per egg to as low as 0.5 mg (70), the latter of which had a slight rickettsiostatic effect. There is little evidence of the activity of streptomycin on these agents *in vitro*. Streptomycin had an additive effect when used with para-aminobenzoic acid or nitroacridine (71). Giroud (72) failed to demonstrate any action of streptomycin on the evolution of disease after the intranasal infection of mice with *R. mooseri*. There was, however, some reduction in number of rickettsiae and a change in their morphology. On the other hand, some 18 to 18,000 units/ml mixed with the rickettsiae had a significant effect on the dermal reaction produced by *R. mooseri* in the skin of rabbits (72).

Dihydrostreptomycin was also tested by Smadel, Jackson and Gauld (71) and was found to have an activity less than that of streptomycin. The latter drug gave a minimal response at 1 mg per egg against *R. rickettsii* and *R. akari* in the hands of these investigators, whereas the minimal effective dose of dihydrostreptomycin was 2.5 mg per egg.

Preliminary work (73) has shown that the vole rickettsia of Baker (*R. microti*, nom. nov.) is also susceptible to streptomycin *in vivo*, but the exact limit of effective concentrations remains to be established. Activity of crystalline streptomycin has also been demonstrated against *C. burneti*, the agent of Q fever (74). In the infected embryo a rickettsiostatic effect was produced with as little as 0.5 mg per egg. In the guinea pig an infective dose producing 95 per cent mortality rate in the controls was used. Subcutaneous administration of 30 mg of streptomycin daily in three to six divided doses protected 79 per cent of the animals, although all showed signs of infection, such as fever.

In the Chlamydozoaceae, tests of streptomycin activity have been reported for *M. felis* (feline pneumonitis), *M. psittacii* (psittacosis), and *M. lymphogranulomatis* (lymphogranuloma venereum). Against the first two agents, streptomycin has no activity *in vivo* in the chicken embryo (75, 76) in the mouse (77) or, finally, *in vitro* (78). The data concerning the activity of streptomycin against *M. lymphogranulomatis* are conflicting. Wall (79) found one sample of streptomycin active *in vivo* in the chicken embryo; the quantities used were not stated. Hamre and Rake (76), on the other hand, were unable to demonstrate any activity of streptomycin *in vivo* or *in vitro* against this agent.

It has not been possible to produce a laboratory infection with the agents of trachoma or inclusion blennorrhea in any animals but monkeys and apes. For this reason, experimental studies with streptomycin in these infections have not been carried out. Bietti (80) has shown however, that streptomycin has no effect on the etiologic agent of trachoma in man, although it

the hamsters against a dose lethal for all the controls. Streptomycin was more than half as effective as penicillin. Levaditi and Vaisman (62) studied mice infected with *B. duttoni* by the intraperitoneal route. Subcutaneous treatment with streptomycin in daily doses of 5,000 units per mouse for 6 days produced complete suppression of the infection. A total dose of 30,000 units was also capable of sterilizing 62 to 78 per cent of brains of chronic carriers of *B. duttoni* 58 to 94 days after infection. Wylie and Vincent (63) also tested streptomycin against many different leptospirae *in vitro*. Five units of streptomycin per milliliter gave some activity against six strains of *L. icterohaemorrhagiae* and one strain each of twenty-three other species or subspecies, including *L. canicola*. In most cases, however, streptomycin was less active, unit for unit, than was penicillin *in vitro*.

Several studies have been made with *T. pallidum*. The first was a clinical study by Herrell and Nichols, (64) who showed that total doses of 1,200,000 to 10,000,000 units per patient administered intramuscularly, subcutaneously, or intravenously over a 10-day period produced disappearance of *Treponema* in 21 to 81 hours. In all cases, however, this response was followed by relapse. Dunham and Rake (65) tested streptomycin in a standardized intradermal infection of rabbits with *T. pallidum*. They found an activity 1/3,000 that of penicillin, and the smallest dose, divided into twenty-four intramuscular injections, which had any effect when administered 72 hours after infection was 79,000 units/kg. Johnson and Adcock (66) also found some activity against intratesticular infection in rabbits; they used 200 mg equivalents for 3 or 4 days in five divided doses intramuscularly. Fisken and Gruhzit (67) found no result on a maximal dose of 4,000 units/kg for 21 days followed by 10,000 units/kg daily for 14 days, showing that even large doses, in this case a total of 224,000 units/kg, are without effect if so divided that adequate blood and tissue levels are not achieved. Drouhet (68) tested the activity of streptomycin against *T. pallidum* in the mouse and found that sterilization of the tissues did not occur until a total dose of 25,000 units—5,000 units a day for 5 days—was reached

In general, then, streptomycin is active against all the spirochetes tested, but the activity is less than that of penicillin and, at least in syphilis, is not high enough to be of practical importance.

Rickettsiales (69)

In the laboratory, streptomycin has been shown to have rickettsiostatic activity *in vivo* against certain members of this order of organisms and none against other members In the Rickettsiae themselves, activity has been found against *R. mooseri* (70–72) (epidemic typhus), *R. prowazeki* (70, 71) (endemic typhus), *R. akari* (71) (rickettsial pox), and *R. rickettsii* (71) (spotted fever) but not against *R. orientalis* (70, 71) (scrub typhus). Most of these

(B) (91)	Sodium penicillin G	2,000 units
	Streptomycin	6,000 units
	Water to make 1 ml	

Dilute specimen with 1 in 1 part of diluent.

Although no activity has been obtained with streptomycin on animal viruses, activity against bacterial viruses has been demonstrated, thus indicating further the fundamental differences between the phages and the animal viruses. Jones (92) showed that streptomycin inactivated two *E. coli* phages and one for *S. aureus* when these bacterial viruses were present in bacterial-cell-free filtrates. In one case (93) the activity on the phage (*E. coli* phage PC) appeared to vary with the preparation of streptomycin, suggesting that the activity might be due to an impurity. Cohen (94), however, obtained inactivation of a similar *E. coli* phage with crystalline streptomycin. The discovery of strains of phage which act on streptomycin-producing strains of *S. griseus* (95) indicate that streptomycin has low activity against some phages.

Cryptococcus

Since streptomycin exhibits only slight *in vitro* activity against the fungi, little attempt has been made to study its effect on experimental infections with fungi. Although *in vitro* titrations indicated slight anticryptococcal activity of streptomycin, Beck and Muntz (96) found the antibiotic to exercise some beneficial effect on experimental infections with *C. neoformans* (*T. histolytica*) in rats. Three daily doses containing a total of 3,000 units of the drug were administered for 3 weeks. After 10 weeks, 61 per cent of the treated and 33 per cent of the untreated animals had survived. Segrétain and Drouhet (97) obtained no protection by treatment of infected mice with 5,000 units of streptomycin daily for 10 days.

Protozoa

The activity of streptomycin has been tested against plasmodia, trypanosomes, and trichomonads. A limited degree of activity has been found against species of the first-named protozoan but none against the other two, and indeed streptomycin with or without penicillin is now recommended for use in isolation procedures to obtain protozoal cultures free from bacteria.

Seeler *et al.* (98) administered streptomycin to White Leghorn chicks or White Pekin ducklings intramuscularly every 3 hours for 3 to 5 days at doses ranging from 25,000 to 400,000 units/kg daily. The chicks had been infected intravenously with *P. gallinaceum* and the Pekin ducklings intravenously with *P. cathemerium* or *P. lophurae*. Streptomycin therapy was begun 1 hour after infection. Streptomycin, even at a dose of 400,000

does have some effect on the disease itself by reason of activity against the secondary bacterial invaders.

Groupé, Winn, and Jungherr (81) recently described an etiologic agent in infectious sinusitis of turkeys which morphologically resembles members of the Chlamydozoaceae. Although the exact relationship of this agent remains to be determined, it is interesting to note that it has been shown to be susceptible to streptomycin. Thus, using a combined *in vitro* and *in vivo*, technic, Groupé (82) demonstrated that 63 units of streptomycin per egg produced significant delay in the death of embryos receiving the virus-drug mixture as compared to the control group. If treatment was carried out by injecting streptomycin into the yolk sac 15 minutes before or 2 days after the infecting inoculum was given, 5,000 units per egg were required to produce significant delay.

Virales

If the agents belonging to the Chlamydozoaceae are excluded from the order Virales—and there is good reason to exclude them and to place them in the order Rickettsiales (69)—then it can be said emphatically that streptomycin has no activity *in vitro* or *in vivo* on any of the animal viruses (83, 84) on which it has been tested, from the poxes (85) (the largest) to the viruses of foot and mouth disease or poliomyelitis (the smallest). So free from any antiviral activity is streptomycin, that it may be used, preferably in combination with other antibacterial substances such as penicillin and the sulfonamides, to sterilize of bacteria materials from which it is hoped to isolate viruses. Among such materials are skin, nasopharyngeal exudates (86, 87), pulmonary tissue and sputum (88), intestinal contents (89), and urine. It has also been employed to advantage as an antibacterial agent in the treatment of calf lymph used in the production of smallpox vaccine. Even against the Rickettsiales, streptomycin is apparently inactive *in vitro* in all cases in which it has been tested. It may, therefore, be used to sterilize similar materials for isolation of these agents, provided it is not used, in the case of those agents against which it is active *in vivo*, in concentrations such as to show activity when such mixtures are inoculated into the fertile egg or other experimental host.

Several mixtures have been suggested for antibacterial activity on body fluids or tissues, among which may be mentioned:

(A) (90)

Tyrothricin	2 mg
Sodium sulfadiazine	50 mg
Streptomycin hydrochloride	25,000 units
Broth pH 7.6 to make 100 ml	

Dilute specimen with 1 in 4 or 9 parts of diluent.

days. Zintel (104) later tested the efficacy of streptomycin alone as compared with combined therapy. He also used dogs. Having ligated the the appendix and the blood supply to it, he opened this organ and smeared the peritoneum thoroughly with the appendiceal contents. Of his control group of dogs, only 6.6 per cent survived. When systemic streptomycin was used, the survival rate was 27 4 per cent. When local sulfanilamide, together with systemic sulfadiazine and penicillin, was used, the survival rate was 40 per cent. When streptomycin was added to the foregoing agents, the survival rate was 60 per cent, and when combined systemic streptomycin and penicillin were used, the survival rate was 70 per cent. In no cases did Zintel (104) give any information as to the dosage schedule employed. Davis *et al.* (105) tested the effect of streptomycin in experimental strangulation of the bowel in rabbits The experimental strangulation was produced by devascularization of approximately 7.5 cm of bowel. In a control group all rabbits were dead by the 19th day. In one group in which 80 mg of streptomycin was given subcutaneously in a single dose daily, starting immediately after operation and continuing for 25 days, 62.5 per cent were dead in 19 days. In a third group of twelve animals receiving 200 mg daily for 7 days and 100 mg daily for an additional 21 days, the total daily dose being divided into two subcutaneous doses and treatment starting immediately after operation, all animals survived for 2 months Five rabbits given 100 mg daily for 4 days prior to the experimental strangulation and then 100 mg daily for a subsequent 25 days survived. In all cases the rabbits weighed approximately 2 kg. Farris and Romack (106) tested the effect of both local and systemic therapy with streptomycin in experimental appendiceal and perforation obstruction in rabbits. In local treatment, 25,000 to 100,000 units were injected into the lumen of the ligated appendix. For systemic treatment 25,000 units were given intramuscularly every 6 hours for sixteen doses Local treatment protected all the rabbits, but in every case the appendix, though unruptured, had increased to five times the normal diameter and auto-appendectomy appeared to have occurred. All cultures were negative. In the group treated systemically, one rabbit died, as did all the untreated control group

It is clear from the foregoing that streptomycin either alone or in combination with other drugs, and particularly with penicillin, has a highly favorable effect in experimental peritonitis.

Effect of Streptomycin on Intestinal Flora and on Nutrition of Animals

From both clinical and experimental studies it is clear that streptomycin given by mouth has a marked suppressive effect on the intestinal flora, as indicated by examination of the bacterial content of the feces. After oral

units/kg daily, showed no suppressive activity against any of the three species. At a level of 400,000 units/kg daily, the partly purified streptomycin used in these experiments did have a slight effect on the sporozoite-induced *P. gallinaceum* infection in the chick. Tonkin (99) tested the activity of streptomycin against tissue cultures of the exoerythrocytic phase of *P. gallinaceum* growing in explants of chicken spleens. She found that streptomycin exerted some activity when used at a concentration of 500 units/ml, which was not toxic for the cells in the tissue culture. A concentration of 200 units/ml was not effective. Bratton (100) was unable to demonstrate any action when he used continuous intravenous treatment with streptomycin over a period of 3 days at a dose of 330,000 units/kg daily. In this case the test infection was *P. lophurae* in White Pekin ducks.

As has already been indicated, Merchant and Soule (101) were unable to demonstrate any activity of streptomycin on the three trypanosomes —*T. brucei*, *T. equiperdum*, and *T. hippicum*—tested in mice or chicken eggs. The mice were given subcutaneously a total dose of 16,000 units of streptomycin, and the embryonated chicken's egg was given 40,000 units of streptomycin injected into the yolk sac. Quisno and Foter (102) found no effect of streptomycin at 25 units/ml for 10 hours at 37°C on *Trichomonas vaginalis*. They reported that higher concentrations of streptomycin for a shorter period also had no effect on the protozoa but gave no further details.

Experimental Peritonitis

Possession of sulfonamides and penicillin has given both the surgeon and the physician such excellent weapons against bacterial infections of the body cavities, particularly the peritoneum, that the mortality rate from peritonitis is no longer so high as it was formerly. Certain organisms, however, have proved highly resistant to these drugs. Against such organisms streptomycin has proved to be of exceptional importance because so many of them belong to the group of gram-negative bacilli against which streptomycin is particularly active.

Murphy, Ravdin, and Zintel (103) were able to obtain a 30 per cent greater survival of dogs that had experimental peritonitis when the animals were treated with streptomycin than when they were untreated. The streptomycin passed from the blood into the peritoneum in appreciable amounts even in normal dogs into whose peritoneal cavity 200 ml of physiologic saline solution had been injected In the experiment, peritonitis was produced by artificial perforation of the ligated appendix. Treatment was with 0.75 mg of streptomycin per pound per hour intramuscularly every 4 hours for 40 hours beginning 1 hour after operation. Subsequently, 25 mg was administered every 4 hours for 2 days and every 8 hours for 3

Such administration of streptomycin, with or without sulfasuxidine, gave rise to marked reduction of coliforms in the cecal contents. There was no evidence of biotin deficiency.

Kane and Foley (110) showed that, in man, as little as 1 gm of streptomycin by mouth daily in two doses, or rectal lavage with 20 ml containing 0.0005 gm/ml caused all viable *E. coli* to disappear from the feces. *E. coli* reappeared within 1 day of cessation of treatment. Fecal streptococci, clostridia, bacteroides, and candida were unaffected. Dalton (111) examined the feces of children receiving treatment with streptomycin. Although in films a fair number of gram-negative bacilli could be demonstrated, in most cases none could be grown. In a few cases, however, a streptomycin-resistant culture of *A. aerogenes* did appear.

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administration of streptomycin, some 95 to 98 per cent can be recovered in the feces (see chapter 14). Because of the increasing evidence that many animals are dependent for certain vitamins and other essential factors on the production of these factors by bacterial fermentation in the intestines, it would be of interest and importance to determine what effect very prolonged oral administration of streptomycin would have on the nutrition of the recipient. Unfortunately, there is no adequate systematic investigation on this point at the present time.

Smith and Robinson (107) fed streptomycin to CFW mice weighing between 18 and 20 gm. The mice were divided into two groups, one of which received a concentration of 250 units of streptomycin per gram of food, and therefore ingested approximately 30,000 units/kg daily; and the other of which received 2,500 units of streptomycin per gram of food and ingested approximately 300,000 units/kg daily. Such oral administration was continued for 21 days. On the lower dosage regimen, the effect was particularly striking during the first 9 days, after which certain groups of organisms, but not the coliforms, began to reappear in the stools despite the continued administration of the drug. Within 24 hours of first administration of the drug diet, the coliform count dropped from 330,000 to 330 per gram of feces and the count of nonlactose fermenters dropped from 330,000,000 to 16,500. Streptomycin proved to be more active than streptothricin, sulfaguanidine, or sulfasuxidine. On the higher dosage regimen, all coliform and gram-negative organisms disappeared within 24 hours, and the stools remained free from these organisms during the whole 3-week period of treatment. Even after administration of streptomycin was stopped, it was 6 days before the feces showed a normal count of gram-negative organisms. During the period of administration of streptomycin, only a few gram-positive sporeforming organisms were found in the feces. Usually, resistance to streptomycin did not develop in any organisms, the only exception to this being certain strains of *S. marcescens*. As might be expected, species most sensitive to streptomycin were eliminated first. Smith and Robinson (107) were unable to demonstrate any evidence of vitamin deficiency, loss of weight, or other signs of toxicity in their mice. Later, Emerson and Smith (108) used weanling rats and mice and fed a diet containing 2,500 units of streptomycin per gram. The rats showed a reduction of the coliform count and signs of biotin deficiency, which were entirely eliminated by administration of biotin. The mice remained normal in all respects. Moore *et al.* (109) fed streptomycin alone, or in combination, to White Leghorn cockerel chicks. Streptomycin was not toxic at 500 units per gram of diet. Singly, or in combination with sulfasuxidine, its administration in the basal diet with supplemental folic acid led to increased growth, perhaps due to inhibition of bacteria utilizing certain vitamins.

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CHAPTER 9

STREPTOMYCIN IN EXPERIMENTAL TUBERCULOSIS

The search for a specific chemical agent that could be used therapeutically against infections due to the tubercle bacillus has been long, and until recently, generally futile The advent of the sulfonamides and of penicillin, which ushered in a new era of therapeutics for many infectious diseases, provided only additional disappointment so far as tuberculosis was concerned. However, the fact that these agents were highly effective in certain other bacterial infections was a factor in the renewal of interest in the search for drugs having antituberculosis possibilities.

The first reported evidence that infections due to the human type tubercle bacillus were vulnerable to the action of specific drugs appeared in 1940 (1). The successful drug was p,p′-diaminodiphenylsulfone-N,N′-didextrose sulfonate (promin; fig 17).[1] This compound is a derivative of 4,4′-diaminodiphenylsulfone, which was first synthesized by Fromm and Wittmann in 1908 (2) After a long series of experiments with promin and several other derivatives of 4,4′-diaminodiphenylsulfone, the maximal potency that can be expected from these compounds was finally established, and their shortcomings as antituberculosis agents were recognized.

Under certain prescribed experimental conditions the more effective sulfones are capable of exerting a measurable and frequently a dramatic effect on the course of experimental tuberculosis of guinea pigs (3).[2] Unfortunately 4,4′-diaminodiphenylsulfone and all of its derivatives effective

[1] Promin was synthesized in 1937 by E. W Tillitson, at that time associated with the Research Laboratories of Parke, Davis & Company, Detroit, Michigan

[2] The effective sulfones that have been subjected to considerable study in experimental tuberculosis include "promin," "diasone," "promizole," and "sulphetrone." Another synthetic organic compound that has proved definitely antagonistic for tubercle bacilli *in vivo* is p-aminosalicylic acid. This substance was first described by Lehmann (4) in 1946 Its value in clinical tuberculosis is now being determined by investigations in different parts of the world

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therapy irrespective of the species of animal used. For obvious reasons the smaller laboratory animals such as guinea pigs and mice have generally been utilized in experimental studies concerning the efficacy of streptomycin against tuberculous infections. Any of the larger species of animals that meet the conditions of pathogenicity desired should be acceptable, however, for studies designed to explore the antituberculosis action of this antibiotic.

Guinea pigs

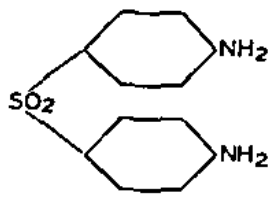
The ability of streptomycin to suppress infections due to tubercle bacilli was first demonstrated in guinea pigs (8). Although the streptomycin used was relatively impure, it was well tolerated in the dosage prescribed, and its capacity to suppress a potentially malignant infection was impressively demonstrated.[5] Previous experience with effective sulfones in experimental tuberculosis in guinea pigs provided a basis for comparative appraisal of the therapeutic potency of streptomycin. The effect of the antibiotic was definite and impressive and indicated clearly the desirability of extending the investigation sufficiently to establish, if possible, the exact status of streptomycin in combating experimental infections induced in guinea pigs by fully virulent mammalian tubercle bacilli. The complexities of the problem were recognized and, while eventually certain important basic facts regarding streptomycin and its efficacy as an antituberculosis agent were elucidated, many additional problems remain. These pertain to (a) optimal dosage of streptomycin; (b) frequency of administration for most satisfactory therapeutic results; (c) optimal duration of treatment; (d) the structural character of the tuberculous process and relationship of streptomycin therapy; (e) the precise role of the resistant or immune state of the host in the final assessment of therapeutic accomplishment; and (f) the exact manner by which the therapeutic action of streptomycin is achieved.

The antituberculosis potential of streptomycin is easily demonstrated in tuberculous guinea pigs by following any of several methods of procedure. The third experiment reported by Feldman, Hinshaw, and Mann (9) illustrates adequately the ability of streptomycin to suppress a formidable tuberculous infection without evidence of serious toxicity. In this experiment the animals were inoculated with the human type of tubercle bacilli (H37Rv) 7 weeks before treatment with streptomycin was begun. Before treatment was started all the guinea pigs were found to be sensitive to a diagnostic dose of tuberculin, administered intradermally. Twenty-five animals were treated with streptomycin; twenty-four were not treated. The daily amount of streptomycin was 6 mg divided into four equal doses

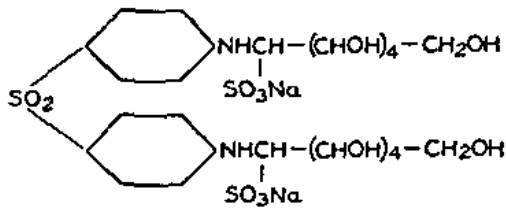
[5] The streptomycin used in the first experiment to determine its possible efficacy in experimental tuberculosis was obtained in April 1944, through the kindness of Dr. S. A. Waksman.

against the tubercle bacillus, *in vivo*, have hemotoxic potentialities. This toxic possibility coupled with the fact that, for human beings, the antituberculosis potential of these drugs in the doses tolerated is of a relatively low order, definitely circumscribed the usefulness of the sulfone group of compounds in treating clinical tuberculosis.[3]

That an antibiotic substance effective against tuberculous infections would eventually become a reality has been suggested by many observations reported during the 60 or more years that antedated the discovery of streptomycin. Chemical substances of microbial origin capable of inhibiting the growth of tubercle bacilli *in vitro* are, as a matter of fact, relatively numerous and of rather common occurrence. At present between 40 and 50 such substances have been described, and every year sees the appearance in the literature of a few to several new ones.[4] Few of the anti-



4 4′-diaminodiphenyl sulfone



Promin

FIG 17. Structural formulas of 4,4′-diaminodiphenylsulfone and p,p′-diaminodiphenylsulfone-N,N′ didextrose sulfonate (promin)

bacterial substances derived from microbes that perform so dramatically *in vitro* have proved of value, however, when used to combat a tuberculous infection.

Of the few antibiotics that have exhibited at least some antituberculosis potency, streptomycin is, up to the present time, by far the most effective and the least toxic.

STUDIES ON ANTITUBERCULOSIS ACTION

So far as is known, experimental tuberculous infections produced by human or by bovine strains of *M. tuberculosis* will respond to streptomycin

[3] It is of interest to know that the therapeutic value of certain sulfones in the treatment of leprosy is widely accepted, the possible value of these sulfones in clinical tuberculosis when used in combination with streptomycin is now being explored.

[4] The literature pertaining to antibiotic agents in tuberculosis has been reviewed by Feldman (5), by Hart (6), and by Waksman (7).

called that before treatment, all of the animals were positive to a diagnostic dose of tuberculin. A second tuberculin test for sensitivity was made at

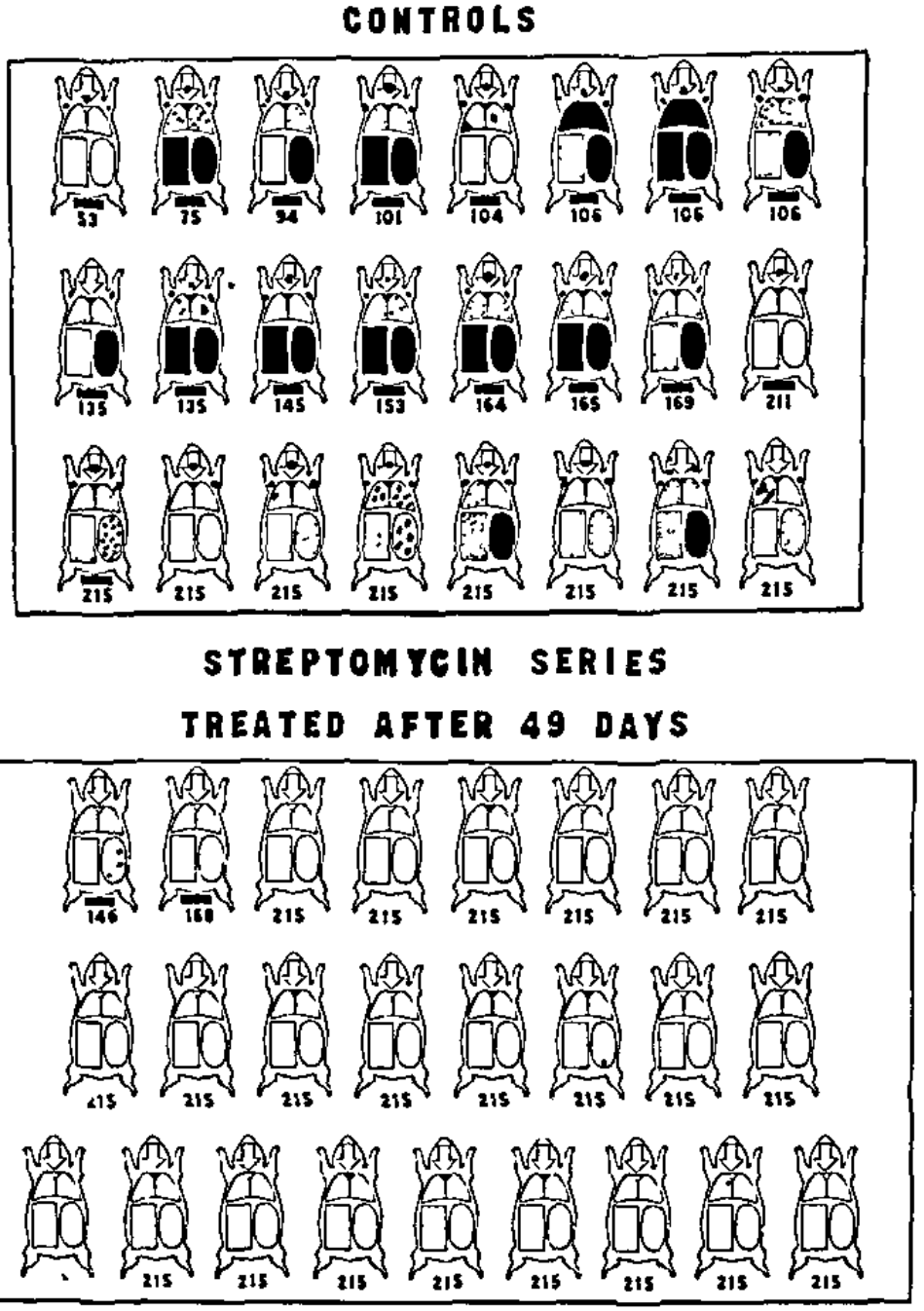


FIG. 19. Schematic representation of amount of tuberculosis noted at time of

the end of the period of medication and nine (39 per cent) of the twenty-three animals that had survived the period of observation were negative to the second test. Furthermore, in seven of the nine animals in which a neg-

and administered subcutaneously every 6 hours. Treatment was continued for 166 consecutive days, and the experiment was terminated 215 days after the animals had been inoculated with tubercle bacilli.

The results of this experiment are illustrated in figures 18 and 19.

The striking difference in the survival rate of the treated and untreated animals is apparent in figure 18. During the period of observation, approximately 70 per cent of the control or untreated animals died while only two (8 per cent) of the treated animals died.

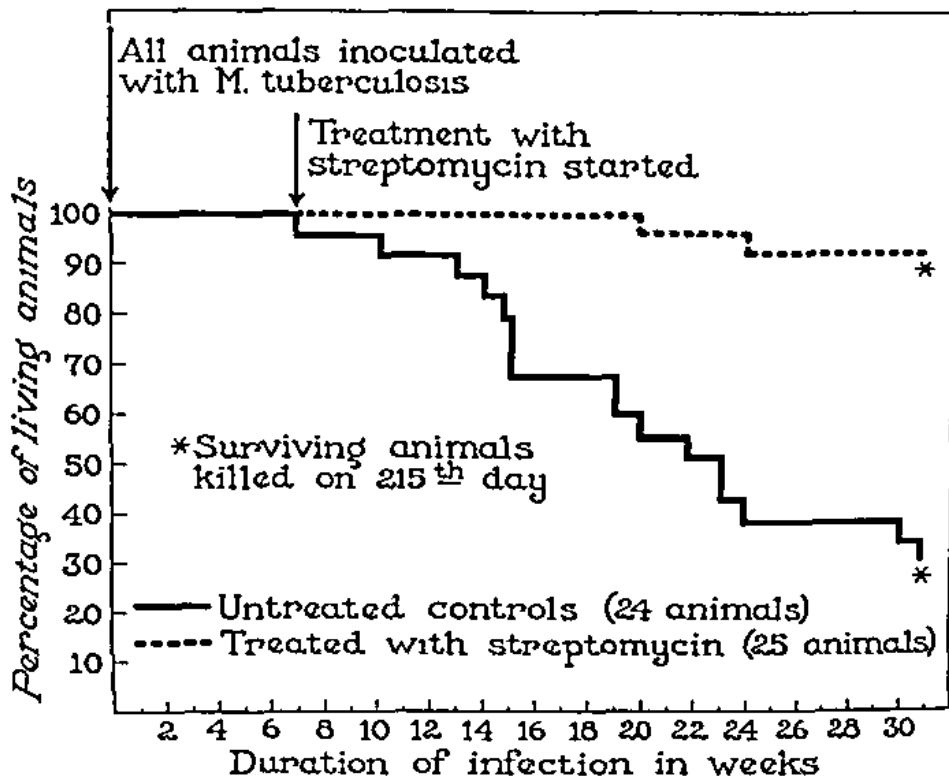


FIG. 18. Comparative mortality rates of tuberculous animals treated with streptomycin and of untreated controls (9).

The antituberculous effect of the treatment was clearly apparent (fig. 19). The severe, widely disseminated tuberculosis among the controls is in dramatic contrast to the small amount of disease indicated among the animals that received streptomycin. When the tissues of the animals that were treated were examined microscopically, 52 per cent of the guinea pigs were devoid of tuberculous lesions and the disease among the other 48 per cent was regressive in character with definite signs of healing or arrest.

In addition to the conspicuous morphologic signs of successful treatment, this experiment yielded other and significant evidence of the antagonistic effect of streptomycin against the infective microorganism. It will be re-

lethal course of the infection have been very few. When dealing with a tuberculous infection established by intravenous inoculation, the difficulties confronting any known or hypothetical therapeutic substance are profound indeed. A more intractable situation in the realm of chemotherapy of experimental tuberculosis is difficult to imagine.

That streptomycin, in spite of a tremendous handicap, did exert a highly favorable influence in the experiment just recounted provides impressive

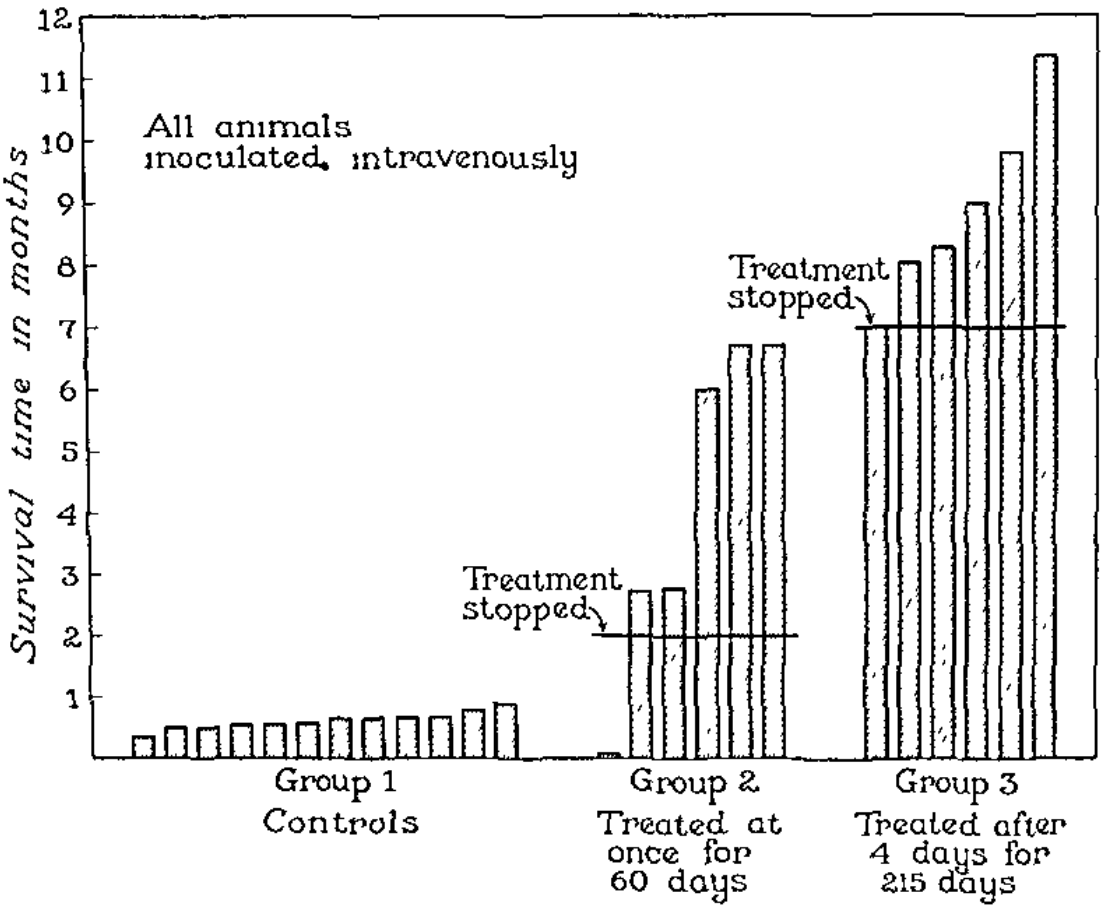


FIG 20. Relative survival time of three groups of guinea pigs inoculated intravenously with 1 mg, moist weight, of tubercle bacilli, strain H37Rv

evidence of the exceptionally high potency of this substance as an antituberculous agent.[6]

Another example of the high potency of streptomycin in experimental tuberculosis is supplied by a report of Steenken and Wolinsky (11), who treated animals previously inoculated intracerebrally. These workers infected guinea pigs by introducing tubercle bacilli (strain H37Rv) directly

[6] In another experiment (10) in which guinea pigs were inoculated intravenously with tubercle bacilli and in which treatment with streptomycin was delayed until the first death occurred (11 days), administration of streptomycin proved ineffective. Apparently the severity and momentum of the disease attained after 11 days of infection constituted a situation that was irrepressible.

ative reaction to tuberculin was recorded, no residual virulent tubercle bacilli could be demonstrated in the spleens by subinoculation into normal guinea pigs.

This reversal of a previously positive to a negative sensitivity to tuberculin in a considerable number of infected animals as a consequence of treatment with streptomycin is indeed unusual. The observation provides at least some presumptive evidence in support of the belief that streptomycin under favorable conditions does eliminate at least some of the infective bacteria. Generally speaking, however, the effectiveness of streptomycin *in vivo* is due to its bacteriostatic rather than to its bactericidal capacities.

From the evidence obtained from the experiment just recounted, several conclusions appear to be justified. The more important of these can be summarized briefly as follows: 1. Streptomycin, under the conditions imposed, was capable of resolving or suppressing potentially lethal experimental tuberculosis established several weeks before treatment was started. Thus was demonstrated the therapeutic rather than the prophylactic potency of this antibiotic. 2. While the anatomic evidence of antagonistic action was undeniable, the major effect on the tubercle bacilli was bacteriostatic rather than bactericidal. 3. The dramatic ability of streptomycin to reverse the potentially malignant course of tuberculosis inoculated into guinea pigs and the absence of signs of serious toxicity even after medication for 166 consecutive days, provided satisfying evidence to justify limited trials of this drug in cases of clinical tuberculosis

The conditions in the experiment just described were made intentionally severe. Unless an antituberculous substance can perform satisfactorily under experimental conditions that are crucial, optimistic expectations for its possible clinical usefulness should not be entertained.

One additional experiment in which the suppressive action of streptomycin in experimental tuberculosis was vividly demonstrated may be referred to briefly (10). Guinea pigs were infected intravenously with 1.0 mg, moist weight, of human type tubercle bacilli. That the infection was exceedingly virulent was revealed by the fact that the first of the twelve untreated animals died 11 days after being inoculated; the last of the untreated controls died on the 27th day. In contrast to the relatively short survival time of the untreated animals was the survival time of those that received streptomycin. As shown in figure 20, most of these lived many months after being infected, the last animal dying 341 days after inoculation.

The significance of this experiment is worthy of brief comment. Intrinsically, the tubercle bacillus and the disease it produces have provided an exceedingly formidable problem for the successful attack by chemical substances. Even under the most favorable experimental conditions, substances that have exerted even a limited favorable effect on the potentially

effect of streptomycin among the vaccinated animals was superior to the effect obtained among the nonvaccinated animals that were treated. It was also observed in most of the animals that were treated that viable, virulent tubercle bacilli were still present after the period of therapy.

From the results obtained in the many experiments with tuberculous guinea pigs a mass of evidence has accumulated attesting to the ability of streptomycin to exert profound and favorable modifications on a disease process that is among the most difficult of the bacterial infections to suppress. The results are reproducible; the facts are convincing, and justify the conclusion that in the experimentally infected tuberculous guinea pig, the effectiveness of streptomycin is unequivocal. It must, however, be borne in mind that the ability of streptomycin *in vivo* to kill tubercle bacilli is definitely circumscribed. Its action in modifying the progress of a tuberculous infection is indirect and related in some obscure manner to interference with the normal pathogenesis of the infective bacteria.[7]

Mice

The use of mice in experimental chemotherapy of tuberculosis has, until recently, received but sporadic attention.[8]

That selected laboratory strains of mice are suitable animals for the investigation of certain phases of tuberculochemotherapy has been well established by the extensive work of Youmans and his collaborators (19–22). Although mice have many desirable attributes for work of this type, the mouse, like all other laboratory animals utilized in the production of experimental tuberculosis, has limitations and shortcomings. These should be recognized when the so-called mouse test is chosen as the procedure of choice in searching for new drugs as antituberculosis agents.[9]

The first report of the ability of streptomycin to suppress a tuberculous infection in experimentally infected mice was by Youmans and McCarter (19). Two experiments were run.

In one of the experiments, thirty white Swiss mice were inoculated in-

[7] Rockwell (13) reported that streptomycin was ineffective in experimental tuberculosis in guinea pigs and rabbits. The daily dose of streptomycin was 14 mg per kilogram. The animals were treated for only thirty-one days. After treatment was discontinued, the animals eventually died and there was present tuberculosis equal in amount to that observed in the untreated controls. Rockwell concluded that streptomycin was ineffective in tuberculosis and that in tuberculosis a drug to be effective must be bactericidal in action.

[8] Important contributions to the knowledge of the pathogenesis of tuberculosis for mice have been made recently by Pierce, Dubos and Middlebrook (14); Raleigh and Youmans (15), Youmans and Raleigh (16); Swedberg (17); and by Martin (18).

[9] Pharmacologic studies of mice after administration of streptomycin have been reported by Rake and Donovick (23).

into the cerebrum. Half of the infected animals were treated with streptomycin starting on the day of inoculation; the remaining animals were untreated controls. With the exception of one untreated animal that succumbed on the 92nd day after infection, all of the untreated controls were dead on the 22nd day after being inoculated.

With the exception of two guinea pigs that died early of intercurrent disease, the animals that received streptomycin lived for rather prolonged periods. It was observed, however, that duration of life was definitely related to the duration of treatment. Animals treated for only 58 days had a mean survival time of approximately 117 days; those that were treated continuously were still living at the time the work was reported (173 days after being infected).

In guinea pigs intracerebral inoculation, like intravenous inoculation, produces a most formidable experimental tuberculosis. Regardless of the unusual obstacle provided by the experimental procedure, results of Steenken and Wolinsky's study indicate that streptomycin was highly effective in suppressing the pathogenesis of the infection and in delaying the lethal outcome of the disease. That the drug is effective in experimental tuberculous infection of the central nervous system is particularly significant.

Experimental streptomycin therapy is also impressively effective in guinea pigs inoculated intraperitoneally with relatively large amounts of tubercle bacilli. The intraperitoneal method of inoculation results in a tuberculous infection only slightly less formidable than that which follows the intravenous or intracerebral procedures. In addition to the involvement of the peritoneal surfaces, severe and widespread dissemination of the infection to the lungs, liver, and spleen is virtually inevitable. Even such a potentially disastrous situation can be favorably and at times dramatically modified by administration of streptomycin

Steenken and Wolinksy (12) investigated the influence which antituberculosis vaccination might have on the suppressive potential of streptomycin in experimental tuberculosis of guinea pigs Vaccinated animals, and for comparison, animals that had not been vaccinated were inoculated with tubercle bacilli (strain H37Rv) 49 days before treatment with streptomycin was started. The daily amount of streptomycin per animal was 24 mg divided into six equal doses, 4 hours apart The duration of treatment varied from 40 to 125 days The results showed that, of the control or untreated animals, all had died between the 69th and the 140th day, whereas all animals that had received treatment were living when the experiment was brought to a close 175 days after the animals had been infected. On the basis of the amount of tuberculosis observed in the treated and in the untreated animals, the therapeutic ability of streptomycin was definitely apparent. Of great interest and significance was the observation that the

effect of streptomycin among the vaccinated animals was superior to the effect obtained among the nonvaccinated animals that were treated. It was also observed in most of the animals that were treated that viable, virulent tubercle bacilli were still present after the period of therapy.

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Mice

The use of mice in experimental chemotherapy of tuberculosis has, until recently, received but sporadic attention.[8]

That selected laboratory strains of mice are suitable animals for the investigation of certain phases of tuberculochemotherapy has been well established by the extensive work of Youmans and his collaborators (19–22). Although mice have many desirable attributes for work of this type, the mouse, like all other laboratory animals utilized in the production of experimental tuberculosis, has limitations and shortcomings. These should be recognized when the so-called mouse test is chosen as the procedure of choice in searching for new drugs as antituberculosis agents.[9]

The first report of the ability of streptomycin to suppress a tuberculous infection in experimentally infected mice was by Youmans and McCarter (19). Two experiments were run.

In one of the experiments, thirty white Swiss mice were inoculated in-

[7] Rockwell (13) reported that streptomycin was ineffective in experimental tuberculosis in guinea pigs and rabbits. The daily dose of streptomycin was 14 mg per kilogram. The animals were treated for only thirty-one days. After treatment was discontinued, the animals eventually died and there was present tuberculosis equal in amount to that observed in the untreated controls. Rockwell concluded that streptomycin was ineffective in tuberculosis and that in tuberculosis a drug to be effective must be bactericidal in action.

[8] Important contributions to the knowledge of the pathogenesis of tuberculosis for mice have been made recently by Pierce, Dubos and Middlebrook (14); Raleigh and Youmans (15), Youmans and Raleigh (16); Swedberg (17); and by Martin (18).

[9] Pharmacologic studies of mice after administration of streptomycin have been reported by Rake and Donovick (23).

travenously with 0.1 mg of human type tubercle bacilli, strain H37Rv. The infected animals were divided into two groups. Twenty-four hours after the animals had been inoculated, treatment of one group with streptomycin was begun; the amount of streptomycin given daily was 0.3 mg divided into four equal doses and injected subcutaneously at 6-hour intervals. The experiment was terminated after 28 days and the surviving

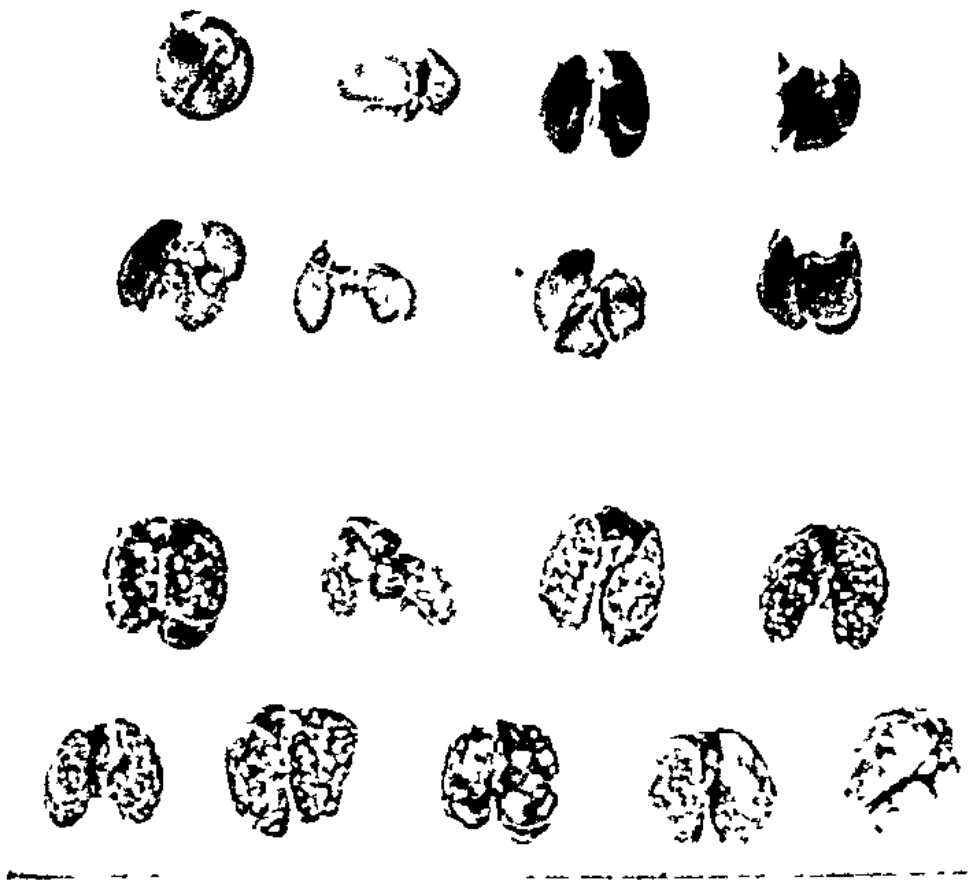


FIG. 21 Lungs from two groups of mice, both of which had been inoculated intravenously with 0 1 mg of streptomycin-sensitive tubercle bacilli. The two bottom rows are from untreated controls, the upper two rows are from mice which received 3 mg of streptomycin subcutaneously daily (20)

animals were killed for necropsy Although some evidence was observed that streptomycin had exerted a favorable effect, the magnitude of the antagonistic action was not impressive The differences that were noted between the treated animals and the untreated controls consisted of a slight prolongation of the mean survival time of the group that received streptomycin, over the mean survival time of the controls, and of a slightly greater extent of lesions and loss of weight in the untreated than in the treated group. These differences, however, were not considered significant, and the experiment was repeated

In the second experiment the procedure was essentially the same as that followed in the first experiment except for the size of the daily dose of streptomycin. The amount of streptomycin administered each 24 hours was 3.0 mg, or ten times the amount given in the previous experiment. At the end of 28 days, when the experiment was terminated, the results indicated definitely that streptomycin had modified the infective process to a marked degree. There was a marked dissimilarity in the mortality rate of the two groups. Almost all the untreated controls had died, whereas of the group

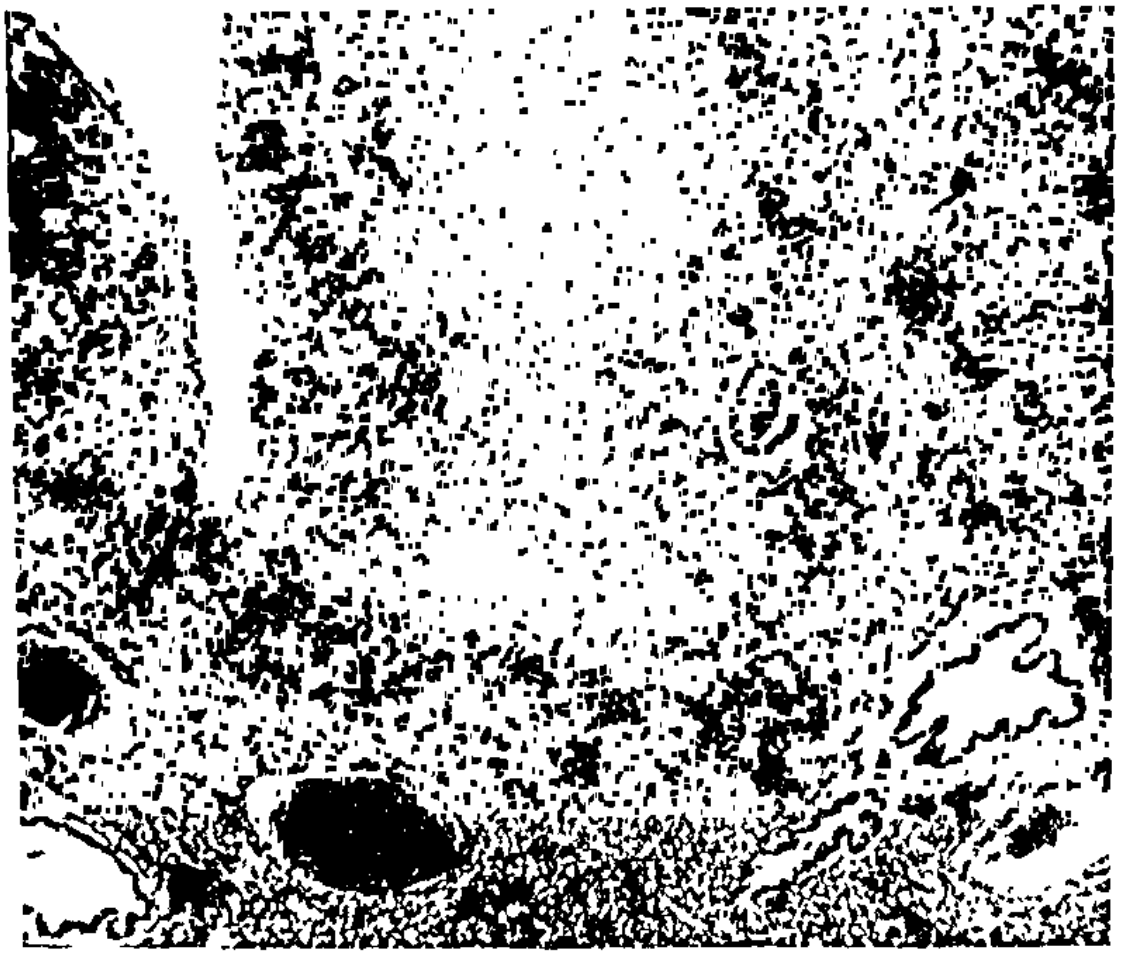


FIG. 22. Lung of untreated tuberculous mouse inoculated intravenously with 0.1 mg of tubercle bacilli, human type (×75). (Courtesy of Dr Guy P Youmans.)

that was treated *only two of fifteen had* died. Of more significance than the difference in the survival rate of the treated and untreated groups of mice was the difference in the amount of tuberculosis present in the two groups. In the controls tuberculous changes occupied 39 per cent, on the average, of the lung substance (fig. 21). Among the animals in the treated group, no lesions were demonstrable grossly, although microscopic lesions were present. Acid-fast bacilli present in the lesions examined microscopically were fewer in the treated than in the control group.

Confirming the results just recounted, Youmans and Raleigh (16) reported two additional experiments on the effect of streptomycin in tuberculous infections in mice. The results show that in the treated animals the infection was effectively suppressed. Further evidences of antagonistic action of streptomycin in the development of tuberculosis of mice were supplied in data reported by Levaditi and Vaisman (24, 25) and by Levaditi, Vaisman, and Lévy (26).

The data reviewed supply impressive evidence that (a) by intravenous

FIG 23 Lung of a tuberculous mouse treated with streptomycin (×75) Compare with figure 22 (Courtesy of Dr Guy P Youmans.)

inoculation, virulent mammalian tubercle bacilli are highly infective for mice, showing a marked and characteristic predilection for the lungs, and (b) the pathogenesis of tuberculosis in mice induced by tubercle bacilli, human type, can be suppressed to a striking degree by administration of daily doses of streptomycin in adequate amounts (figs. 21, 22 and 23).

Albino rats

The albino rat is generally considered to be highly resistant to experimental infection with tubercle bacilli. However, by intraperitoneal or

intravenous inoculation of virulent tubercle bacilli of human or of bovine type a definite and characteristic tuberculous infection can be established. The effect of streptomycin on such an infection was reported by Smith, McClosky, and Emmart (27). A group of twelve albino rats were each inoculated intraperitoneally with 5 mg of human type tubercle bacilli. Treatment with streptomycin was begun on the day the animals were inoculated and continued once daily for 35 days. The daily dose of streptomycin was 50 mg/kg. A similar group of untreated controls were also observed. Starting on the 28th day after infection, animals in both groups were killed at intervals of 1 to 4 days. The last animal in each group was killed 53 days after inoculation. Portions of the lungs of each animal were used for preparing cultures for tubercle bacilli and for the inoculation of guinea pigs. Smears from various tissues were stained appropriately and examined for acid-fast bacilli. The results, though not striking, did indicate that streptomycin had exerted a favorable influence of slight degree on the course of the infection.[10]

Doses and Frequency of Administration

In treating experimental tuberculosis there has been considerable diversity of opinion concerning the size of the optimal daily dose. Likewise, the frequency of administration of the drug has been subject to wide variation.

The known antibiotic substances are excreted relatively rapidly from the body. This fact has made it necessary to administer these substances at frequent intervals if the maintenance of concentrations in the blood of amounts considered adequate for therapeutic results is to be ensured. The presumed necessity for maintaining so-called adequate blood levels in therapy with antibiotic substances is by no means established, however, and the practice of administering such drugs every few hours is based largely on empirical grounds. Certainly in the case of tuberculosis, the necessity of the maintenance of appreciable concentration in the blood to insure therapeutic effectiveness may well be questioned. Evidence in support of this was obtained from an experiment published previously (28).

In the experiment referred to, four groups of guinea pigs, inoculated 23 days previously with 0.1 mg of human type tubercle bacilli (H37Rv), were used to determine the effects of different dosage regimens of streptomycin. The total amount of streptomycin administered was the same for each an-

[10] This experiment, which is only partly recounted in this review, is of considerable interest because of the results obtained in another group of infected rats treated with streptomycin and promin. Though treatment with promin alone failed to exert a significant suppression of the infection, the combined use of streptomycin and promin was reported as favorably modifying the disease.

mal that lived for the duration of the experiment. In three of the groups the daily dose of the drug was 8 mg. The dose schedule for these groups was as follows: one group received the daily dose in a single injection, in another group the daily amount was given in two equal doses, 12 hours apart; in the third group the drug was given four times daily at 6-hour intervals. In the fourth group the daily dose was doubled, a fourth of the dose being administered every 6 hours, but the animals were treated only alternate weeks. The period during which the animals were treated was 60 days.

The results of this experiment indicated definitely that frequent administration of streptomycin during each 24 hours is not essential to successful therapeutic results. Suppression of the disease in all groups was quite comparable whether the drug was given every 6 hours or once every 24 hours. In all groups the suppression of the disease was striking and consistent. Most impressive was the effectiveness of treatment in the group of animals that were treated only every other week.

As a consequence of the results obtained in the experiment just mentioned, we routinely administer the daily dose of streptomycin in one single injection. The results have been entirely satisfactory.

Corper and Cohn (29) have also reported on the influence of the frequency of administration on the ability of streptomycin to suppress the progression of experimental tuberculosis in guinea pigs. The infection was induced by inoculating the animals intravenously with 1 mg of a virulent strain of tubercle bacilli, human type Although the experiments reported by Corper and Cohn differed in several important respects from the experiment referred to previously (28), there was essential agreement regarding the basic question of whether streptomycin must be administered at frequent intervals to exert effective action. It was believed that in treatment with streptomycin "there is a threshold of remote sustained action" and that the effectiveness of streptomycin is not appreciably diminished if the drug is administered infrequently. However, the length of the period of treatment with streptomycin and the phase of the infective process present when treatment is started are important factors that influence the ultimate therapeutic results (30). To be effective, treatment which starts immediately after the animals are inoculated with tubercle bacilli must be continued for 6 to 8 weeks. A shorter period of therapy—10 days—during the earlier phase of the infection has little deterrent effect on the disease (31). It has been our observation that when treatment of tuberculosis induced subcutaneously in guinea pigs is delayed for 21 days after the animals are inoculated, appreciable signs of suppression of the disease may be observed after the animals have received the drug for only 3 weeks. These facts should be borne in mind when considering *in vivo* experiments with streptomycin and tubercle bacilli.

The daily dose of streptomycin used in combating experimental tuberculosis in guinea pigs has varied widely. In our work we have used 6 mg for animals with an average weight of 500 gm at the time treatment started. Other workers have used daily doses of 5 mg (32), 10 mg (31), 11 mg (33),[11] 24 mg (12), and 25 mg (29).

In an attempt to determine the subeffective dose of streptomycin in experimental tuberculosis of guinea pigs, we conducted a series of experiments in which the daily dose varied over a wide range (34). The therapeutic effects of daily doses of 0.1, 0.5, 2, 4, 6, and 20 mg were observed in guinea pigs weighing 500 to 600 gm each. The antituberculosis effects of 20, 6, and 4 mg were of equal magnitude. Each of these dose regimens was sufficiently effective to bring about a striking suppression of the infection. A daily dose of 2 mg, however, resulted in only partial retardation of the disease, and the results with the lowest doses—0.5 and 0.1 mg—were markedly inferior. Although exceptionally small doses of streptomycin are ineffectual in bringing about appreciable modification of an induced tuberculous infection, it does not follow that the antituberculosis efficacy of the drug is directly proportional to the amount of the daily dose. There apparently exists in tuberculous infections a situation which limits the antagonistic action of streptomycin beyond that which may be expected from the minimal effective dose.

In mice inoculated intravenously with human type tubercle bacilli, an effective daily dose of streptomycin was determined by Youmans and McCarter (19) to be 3 0 mg A daily dose of 0.3 mg per mouse was found to be inadequate In tuberculous rats Smith, McClosky, and Emmart (27) gave rather large doses of streptomycin (50 mg/kg), which were apparently well tolerated.

Tuberculous Infection of Developing Chick Embryo

Emmart (35) reported on the tuberculostatic action of streptomycin in the development of tubercles on the inoculated chorio-allantois of chick embryos. Several experiments were performed, and the antagonistic action of the streptomycin was demonstrated by the size and number of the tuberculous foci in the treated materials compared to the extent of involvement of the untreated tissues.

Using a procedure different from that followed by Emmart (35), Lee and Stavitsky (36) demonstrated the suppressive action of streptomycin on chick embryos inoculated intravenously with human type tubercle bacilli. The infected embryos were exposed to streptomycin by placing the anti-

[11] In the paper referred to, the dosage was 40 mg/kg Assuming that the guinea pigs used weighed approximately 275 gm each, 11 0 mg would be the daily amount of streptomycin administered

biotic in solution on the allantoic membrane. The experiment was terminated on the 20th day after inoculation, and the livers of untreated controls and of the embryos that received streptomycin were examined histologically. An average of ten lesions per section was recorded for the untreated group, whereas, with one exception, no lesions were found in the livers from the embryos that had received streptomycin.

The method followed by Lee and Stavitsky appears to offer impressive possibilities for "screening" new compounds for the detection of possible antituberculosis potential.

Streptomycin-resistant Tubercle Bacilli

The clinical implications of drug resistance which a pathogenic microorganism may exhibit *in vitro* are obvious. When it was learned that tubercle bacilli may become resistant to streptomycin as determined by *in vitro* methods, it became necessary to learn whether they were capable of producing progressive tuberculous disease in experimental animals and whether such infections were refractory to streptomycin therapy or other chemotherapeutic agents. It was also of considerable interest as well as practical importance to learn whether the characteristic of streptomycin resistance was permanent or whether it was altered by animal passage, frequent subculture, or prolonged storage.

The regular occurrence of streptomycin-resistant tubercle bacilli in a certain percentage of patients being treated with this antibiotic is now a well-known phenomenon and has been the subject of many papers since Youmans *et al* (37) first reported its occurrence in the early clinical trials of the drug In experimental tuberculosis, however, there have been few reports on the occurrence of streptomycin-resistant tubercle bacilli from treated animals

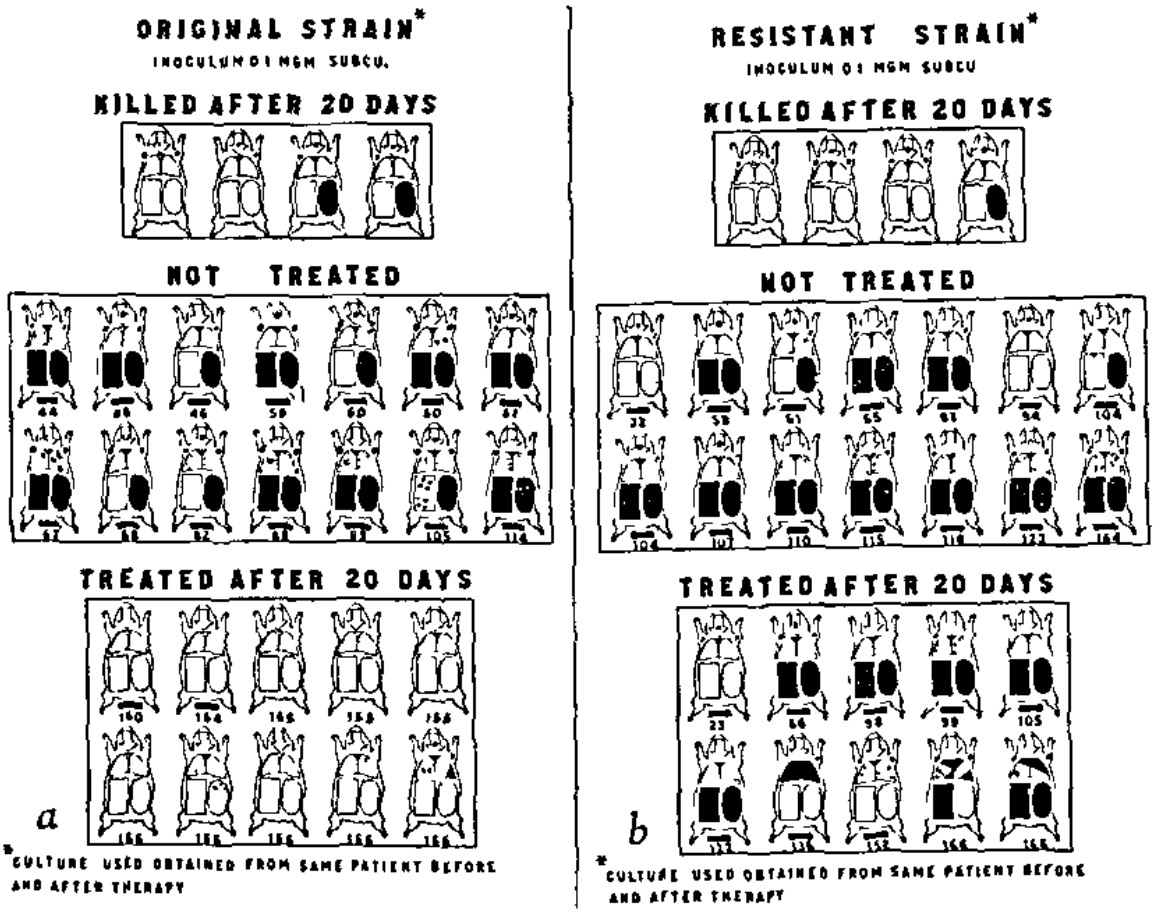
Incidence in guinea pigs and mice

Steenken (38) has reported that streptomycin-resistant tubercle bacilli occur less frequently in treated guinea pigs than in human patients. In his experiments no resistant forms could be recovered from guinea pigs even after 125 days of treatment Our data also indicate that, in guinea pigs, streptomycin-resistant strains are not found in so great a percentage of cases as in human patients In guinea pigs they appear only after prolonged treatment.

We have examined cultures from 103 guinea pigs infected with streptomycin-sensitive tubercle bacilli and treated with 6 mg of streptomycin daily. Only eight cultures have been found to be resistant. Of these three were from animals treated 144 days and five were from animals treated 215 days. Resistant strains could not be found in cultures from sixty-one

animals treated for an average of 50 days or in cultures from thirty-four animals treated for an average of 111 days.

In an experiment described by Feldman *et al.* (39), eight cultures were obtained from the spleens of ten animals infected with a streptomycin-sensitive strain and treated for 144 days with 6 mg of streptomycin daily



in vitro to more than 2,000 micrograms of streptomycin per milliliter of medium. The respective strains of bacteria used for these experiments were obtained from the same patient. That used in experiment *a* was isolated before the patient had received streptomycin, that used to inoculate the animals in experiment *b* was obtained after the patient had received streptomycin for several months. The black bar indicates that the animal died prematurely, the numeral represents the number of days the animal had been infected (39).

(fig. 21*a*). Three of the eight cultures were found to be resistant *in vitro* to more than 2,000 μg of streptomycin per milliliter of medium, whereas the other five were as sensitive as the original culture used for inoculation. In another experiment Feldman *et al.* (10) found that the cultures from one guinea pig treated for 206 days and from four guinea pigs treated for 215 days were all resistant *in vitro* to more than 2,000 μg of streptomycin

per milliliter of medium. In this experiment six animals were infected intravenously with a sensitive strain, and treatment with 6 mg of streptomycin daily was started in 4 days (fig. 20). Treatment was discontinued after 215 days. One animal had died on the 206th day of therapy. All the other treated animals eventually died after treatment was discontinued. The first one died 30 days after discontinuance of treatment, and the last one died in 126 days. Positive cultures were obtained in five of the six, and each culture was resistant.

Youmans *et al.* (40) recently reported that resistant strains may be recovered from mice inoculated with sensitive strains and treated for prolonged periods. In a group of nineteen mice treated with 1.5 mg of streptomycin daily for an average of 131 days, seventeen (89 per cent) had resistant strains. A second group of twenty infected mice were treated daily with 0.75 mg of streptomycin for an average of 73 days. Resistant strains were recovered from thirteen (65 per cent). Other groups treated for shorter periods with smaller doses of the drug had a much lower percentage of animals with resistant strains. Lenert and Hobby (41) have also reported the finding of resistant as well as streptomycin-dependent strains in treated mice.

Permanence of resistance

Although the era of streptomycin therapy has not been long enough to make it possible to examine streptomycin-resistant cultures more than 2 or 3 years old, we do have evidence that, in the case of tubercle bacilli, resistance is a permanent characteristic and that it is not altered by animal passage, repeated subculture, or storage.

Middlebrook and Yegian (42) found that a culture of human type tubercle bacillus (H37Rv) which was rendered resistant to 1,000 μg of streptomycin per milliliter of medium by growing it in the presence of the drug retained its resistance for at least 4 months after being subcultured many times. Youmans and Williston (20) reported that cultures isolated from mice infected with streptomycin-resistant tubercle bacilli were as resistant as the original culture.

We have reported (43) that streptomycin-resistant tubercle bacilli in sputum, urine, or gastric washings will still be resistant when isolated from guinea pigs 8 to 10 weeks after injection with these materials. We have extended these observations and have found that in each of forty-two instances in which such tests were made the cultures isolated from guinea pigs inoculated with sputum or other material from treated patients were as resistant as the cultures made directly from a portion of the same material. In addition we have examined cultures from eighty-three guinea pigs inoculated with resistant strains and have found each to be as resistant as the culture used for inoculation.

Streptomycin-resistant cultures retain their ability to grow in the presence of high concentrations of streptomycin after repeated subculture as well as storage for many months on egg-yolk-agar medium. Seventeen resistant cultures on egg-yolk agar were stored in the refrigerator for 16 months. Only seven were found to be viable at the end of that period. Each of these was as resistant to streptomycin as when stored. We have four cultures that have retained their resistance for 3 years and twenty-two that are 2 years old, during which time they were subcultured on streptomycin-free medium about every 3 months. The permanence of streptomycin resistance lends support to the belief that the characteristic is a genetic mutation instead of an adaptation.[12]

Virulence of streptomycin-resistant tubercle bacilli

A review of the work done by others and of our own experience shows that streptomycin-resistant tubercle bacilli are capable of producing in mice and guinea pigs a progressive tuberculous disease. The culture of the human strain (H37Rv) which Middlebrook and Yegian (42) found to be resistant to streptomycin after exposure to the antibiotic *in vitro* was able to produce in guinea pigs as extensive disease as the sensitive parent strain. Youmans and Williston (20) infected groups of mice intravenously with streptomycin-sensitive and streptomycin-resistant cultures respectively and concluded that they were equally virulent for these animals.

Feldman *et al.* (39) infected one group of ten guinea pigs with a strain of tubercle bacilli sensitive to less than 1.0 μg of streptomycin per milliliter of medium and a similar group with a strain resistant to more than 1,000 μg/ml. These two cultures had been isolated from the same patient. Grossly and microscopically the extent and character of the disease in the two groups were comparable (fig. 24*b*). The disease produced by the streptomycin-resistant strain presented all the features of the typical rapidly progressive disease characteristic of tuberculosis produced in guinea pigs by a virulent human type tubercle bacillus. The group of ten animals infected with the sensitive strain had an average survival time of 70.5 days as compared to 95.5 days for the ten animals infected with the resistant strain.

The evidence from the experiment recounted in the preceding paragraph suggested that in guinea pigs infections induced by streptomycin-resistant tubercle bacilli may proceed at a slower rate than infections due to sensitive strains.

In another study we have used the same strains as in the foregoing ex-

[12] A procedure for the determination of resistance to streptomycin by the use of egg yolk-agar has been described (44).

periment, inoculating each of twenty guinea pigs with 0.1 mg of the sensitive strain and twenty with the resistant strain. In the group infected with the sensitive strain, the first death was recorded on the 39th day, and by the 68th day seven more animals had died. In the group infected with the resistant strain there were no deaths until the 68th day. At the end of 136 days, when the experiment was terminated, there were only two survivors in the group infected with the streptomycin-sensitive strain as compared with eleven survivors in the group infected with streptomycin-resistant tubercle bacilli. The average survival time for the eighteen that died in the first group was 80 days as compared with 103 days for the nine that died in the second group.

A similar comparison was made with a sensitive and a resistant strain from another patient. Nineteen guinea pigs infected with the sensitive strain and fifteen infected with the resistant strain were available for a comparison of the course of the infection At the end of 174 days of infection, seventeen (89 per cent) of those infected with the sensitive strain were dead as compared to eleven (73 per cent) of those infected with the resistant strain. The average survival time for those that died in the two groups was 120 and 133 days respectively

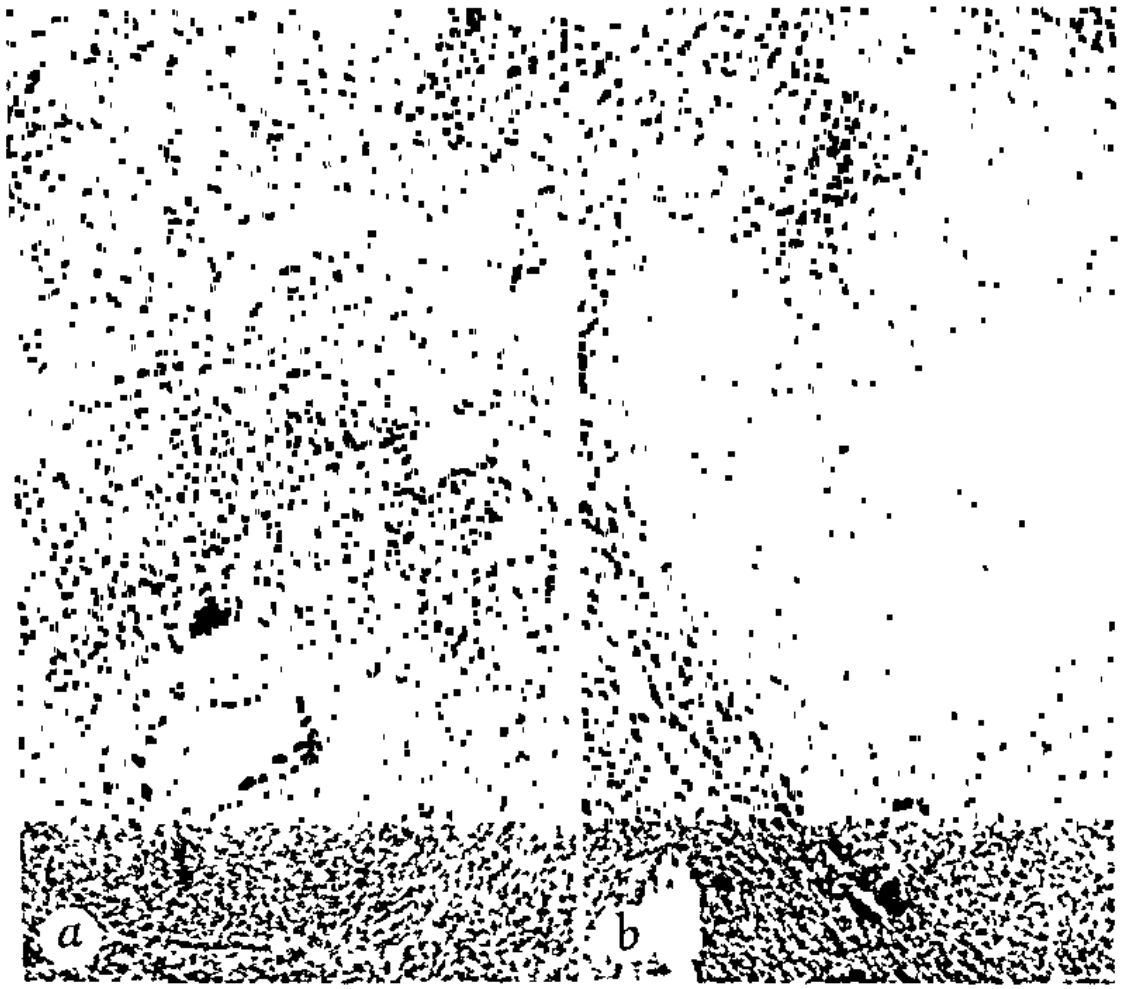
These limited observations suggest that streptomycin-resistant strains of tubercle bacilli, though still capable of producing progressive disease, do so at a slower tempo than do the streptomycin-sensitive strains

Effect of resistance on response to treatment

It has been shown that experimental tuberculosis in mice and in guinea pigs caused by streptomycin-resistant strains is refractory to streptomycin therapy. Youmans and Williston (20) found that streptomycin therapy had no suppressive effect on tuberculosis induced in mice by strains of tubercle bacilli resistant *in vitro* to more than 1,000 μg of streptomycin per milliliter of medium This was true for a strain of tubercle bacilli that had become resistant after exposure to the drug *in vitro*, as well as for a strain that had been isolated from a patient treated with streptomycin.

Feldman *et al* (39) found that streptomycin is not therapeutically effective in guinea pigs infected with a resistant strain of tubercle bacilli In this experiment (fig 24 *a* and *b*) one group of guinea pigs was infected with a strain of tubercle bacilli sensitive *in vitro* to less than 1.0 μg of streptomycin per milliliter of medium A similar group was infected with a strain resistant *in vitro* to more than 2,000 μg/ml These strains had been isolated from the same patient before and after a course of streptomycin therapy respectively As shown in figure 24*b*, the disease in the animals infected with the resistant strain did not respond to treatment, and the extent and the character of the disease in the controls and in the treated

animals were comparable. Cultures isolated from these animals were found to be as resistant *in vitro* as the original culture. The disease in animals infected with the sensitive strain responded in the expected favorable manner except in three animals (fig. 24*a*) that had active lesions of recent origin. Tubercle bacilli resistant to more than 2,000 μg of streptomycin per milliliter of medium were isolated from these three animals.



ve tubercle tissues tu- ograms per ly inactive hown in *a*, s which are

calcified (×70) (39)

Histologic examination of tissues revealed healed lesions and also active lesions of recent origin (fig. 25*a* and *b*). It is probable that the healed calcified lesions may have been due to the sensitive strain inoculated originally and which was controlled by the treatment and that the recent active processes may have been due to a resistant strain which appeared during the course of the treatment.

Recently Spendlove *et al.* (45) described a streptomycin-resistant culture of tubercle bacilli the growth of which in artificial medium is enhanced by the presence of streptomycin. Although this enhancement effect is lost after four or five subcultures, preliminary studies in guinea pigs indicate that infections produced by this strain progress more rapidly when the animals are treated with streptomycin (46) than when they are not treated.

Lenert and Hobby (41) succeeded in getting 184 positive cultures from 196 mice which had been infected with the H37Rv strain of tubercle bacilli and treated with streptomycin. From thirteen of these animals both streptomycin-sensitive and streptomycin-dependent strains were isolated.

Six of these dependent strains, which required streptomycin for growth, were tested in mice for their ability to produce disease. It was found that only one was capable of causing death at the same rate as the original strain. The other five had very low disease-producing potentials. Administration of streptomycin to mice infected with these dependent strains did not increase the virulence of the strains. Streptomycin-dependent and streptomycin-resistant strains were recoverable from these animals when death finally occurred.

Streptomycin Used Concomitantly with Other Antituberculosis Agents

Administration of streptomycin simultaneously with some other drug which is also effective in experimental tuberculosis was first reported by Smith and McClosky (33) Since their report a number of investigators have turned their attention to this approach to obtain more effective therapeutic results than may be realized with streptomycin alone. Since the known substances effective against tuberculous infections are relatively few, the possible combinations with streptomycin are not many. So far, the reports have dealt with combined treatment with streptomycin and one other drug. The addition of two or more drugs to the streptomycin regimen has, up to the present time, not been reported

The drugs used concomitantly with streptomycin have been promin, (27, 33), diasone (47), the N-propyl and the succinimido derivatives of 4,4′-diaminodiphenylsulfone (48), sulfadiazine (48), 4,amino-4′-galacturonylaminodiphenylsulfone (49), p-aminosalicylic acid (50–52), sulphetrone (32), and potassium iodide (53)

Most of the reports pertaining to results of combined treatment with streptomycin and some other antituberculosis drug indicate that the suppressive action of the two drugs in combination is greater than that of either when used singly

In our approach to the problem of antituberculosis therapy by drugs

used concomitantly, we follow a procedure somewhat at variance with that used by most of those who have reported previously. We agree with Youmans, Youmans, and Osborne (50) that in studies of this kind the daily amount of streptomycin administered should be reduced to an amount that when used alone will be only partly or incompletely effective.

One possibility of the effect of streptomycin used concomitantly with other drugs that has not been stressed in the treatment of tuberculous infections is the effect such combinations may have on the emergence of streptomycin-resistant variants of tubercle bacilli. Because of the difficulties of developing consistently streptomycin-resistant populations of tubercle bacilli in the experimental animal, this phase of the problem has received but slight attention in experimental tuberculosis.[13] Clinically, however, this is now being investigated by our associates, Drs. Hinshaw, Carr, and Pfuetze.

Comment

Drugs considered for their possible value in clinical tuberculosis must first be subjected to crucial conditions of testing against experimental tuberculous infections in animals. Results from *in vitro* tests, regardless of how dramatic or promising they may appear, cannot be accepted as justification for clinical trial of a drug in lieu of results from animal experimentation. For protection of the patient, drugs considered for use in human tuberculosis must have performed satisfactorily in preclinical trials in experimental animals. Such drugs must have demonstrated their ability to suppress a well-established tuberculosis induced by inoculation with a fully virulent strain of human type tubercle bacilli. An effective drug will retard the pathogenesis of the infection and permit the resolution and healing of tuberculous lesions.

The evidence obtained from the many studies of the effect of streptomycin on the natural course of experimental tuberculosis in animals amply justifies the conclusion that this antibiotic is a highly potent antagonistic agent for tuberculous infections in animals produced by fully virulent tubercle bacilli of human or of bovine type.

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[13] As mentioned previously, recent studies by Youmans, Williston, and Osborne (40) have demonstrated the feasibility of inducing streptomycin-resistant tubercle bacilli in a high percentage of mice as a consequence of prolonged treatment with streptomycin.

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tomycin therapy. The strain isolated before treatment was sensitive to 1 μg of streptomycin per milliliter, but the one recovered on the 29th day of treatment was able to grow in the presence of 7,500 μg/ml.

From the blood of a patient with septicemia, Edwards and Kirk (4)

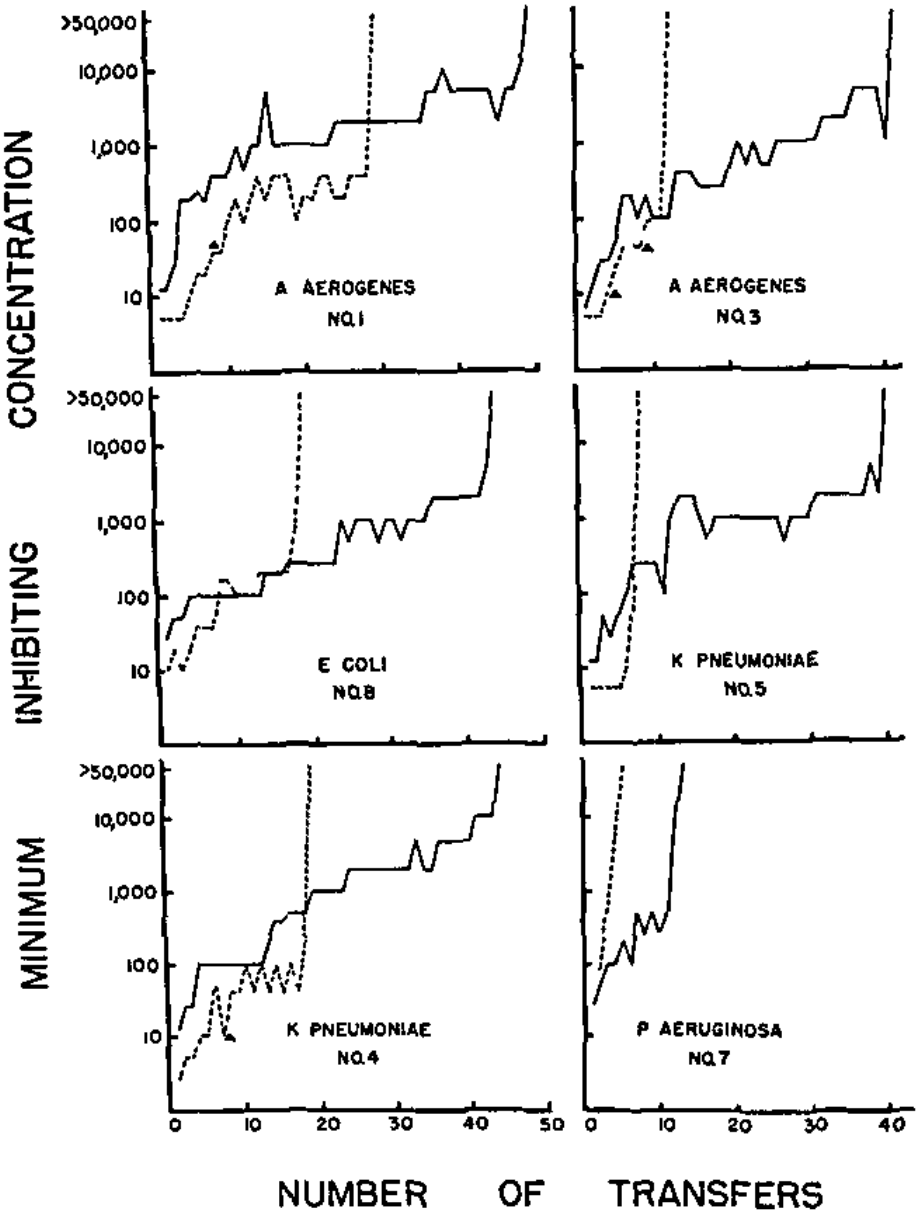

FIG 26 Development of streptomycin resistance during daily transfers in broth (*solid lines*) and on agar (*dotted lines*). A solid triangle ▲ indicates the appearance of a single colony of highly resistant organisms. (After Murray, Kilham, Wilcox and Finland (7))

isolated *A. cloacae* which before treatment was sensitive to 0.1 μg/ml, but which showed in three antemortem and the autopsy cultures progressive increase in streptomycin resistance to a level of 750 μg/ml.

Paine, Murray, Seeler, and Finland (5) reported the development of streptomycin resistance in one strain each of *H. influenzae*, *Ps. aeruginosa*

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CHAPTER 10

DEVELOPMENT OF STREPTOMYCIN-RESISTANT AND STREPTOMYCIN-DEPENDENT BACTERIA

At the time streptomycin was discovered, the problem of bacterial resistance to antibiotics and other chemotherapeutic agents was engaging the attention of a number of investigators It is not surprising, therefore, that reports on the development of streptomycin resistance should have begun to appear soon after the drug was made available for clinical trial and laboratory experimentation. These reports indicated that bacteria developed resistance to streptomycin much more quickly than to penicillin or to other antibiotics, a fact of practical importance to clinicians and of theoretical significance to bacteriologists, particularly to those interested in bacterial genetics.

DEVELOPMENT OF BACTERIAL RESISTANCE

Clinical observations

Development of bacterial resistance to streptomycin as the cause of therapeutic failure was first demonstrated in the treatment of infections of the urinary tract. Finland, Murray, Harris, Kilham, and Meads (1) found that failure of treatment in eight of twelve cases of such infections was associated with rapid development of a high degree of resistance to streptomycin by the causative microorganism. Such observations have since become so common that the view is generally held among clinicians that unless sterilization of the urinary tract is accomplished within the first 2 or 3 days of streptomycin therapy, no beneficial effect can be expected.

Finland *et al.* also noted that resistance was less likely to develop if the urine was kept alkaline This is presumably due to the decreased effectiveness of streptomycin in acid media (2), although no one has investigated the possibility that H-ion concentration may influence the rate of development of resistance *in vitro.*

Hall and Spink (3) observed a patient with subacute bacterial endocarditis due to *B abortus* that became resistant during the course of strep-

and *E. coli communior* during treatment of patients suffering from meningitis.

Buggs and his associates (6) reported a number of clinical cases of various infections in which the bacteria isolated after streptomycin therapy were more resistant than before.

Laboratory studies

In an *in vitro* study of nine strains isolated from urinary infections before treatment, Murray, Kilham, Wilcox, and Finland (7) found that resistance developed either gradually or suddenly, depending upon the size of inocula used. Daily transfers were made in liquid media containing graded concentrations of streptomycin to determine the minimum inhibiting concentration. As shown in figure 26, inoculation of 100,000 to 150,000 bacilli in broth resulted in a gradual, step-wise increase in resistance not unlike the rate at which penicillin resistance develops. Sooner or later, however, a sudden increase occurred, which raised the streptomycin resistance to a very high level. When inocula of several billion microorganisms were transferred daily to streptomycin agar, this sudden rise in resistance always occurred much earlier in the series.

These results resemble those of the earlier experiments of Miller and Bohnhoff (8), who compared the rate of development of resistance to streptomycin and to penicillin by meningococci and gonococci during serial subcultivation on blood agar containing increasing concentrations of the drugs. Both microorganisms developed resistance much more rapidly to streptomycin than to penicillin. After two or three transfers on streptomycin-containing media, abundant growth occurred on concentrations of streptomycin as high as 50,000 μg/ml.

RESISTANT VARIANTS

An explanation of this rapid increase in streptomycin resistance was supplied by the observation (9) that a few colonies of streptomycin-resistant meningococci would develop from a sensitive culture inoculated on solid media containing high concentrations of streptomycin, provided the inocula contained sufficient numbers of organisms. These colonies, indicated by arrows in figure 27, consisted of meningococci which were highly resistant to streptomycin *in vitro* and *in vivo*, although they developed from inocula of a sensitive strain during its initial exposure to streptomycin. They were designated *type A* to distinguish them from the streptomycin-dependent variant (*type B*) discussed later in this chapter.

The streptomycin-resistant variants occurred in cultures of each of eighteen strains of meningococcus which included types I, II, and II alpha (10). In most experiments their numbers were approximately equal on

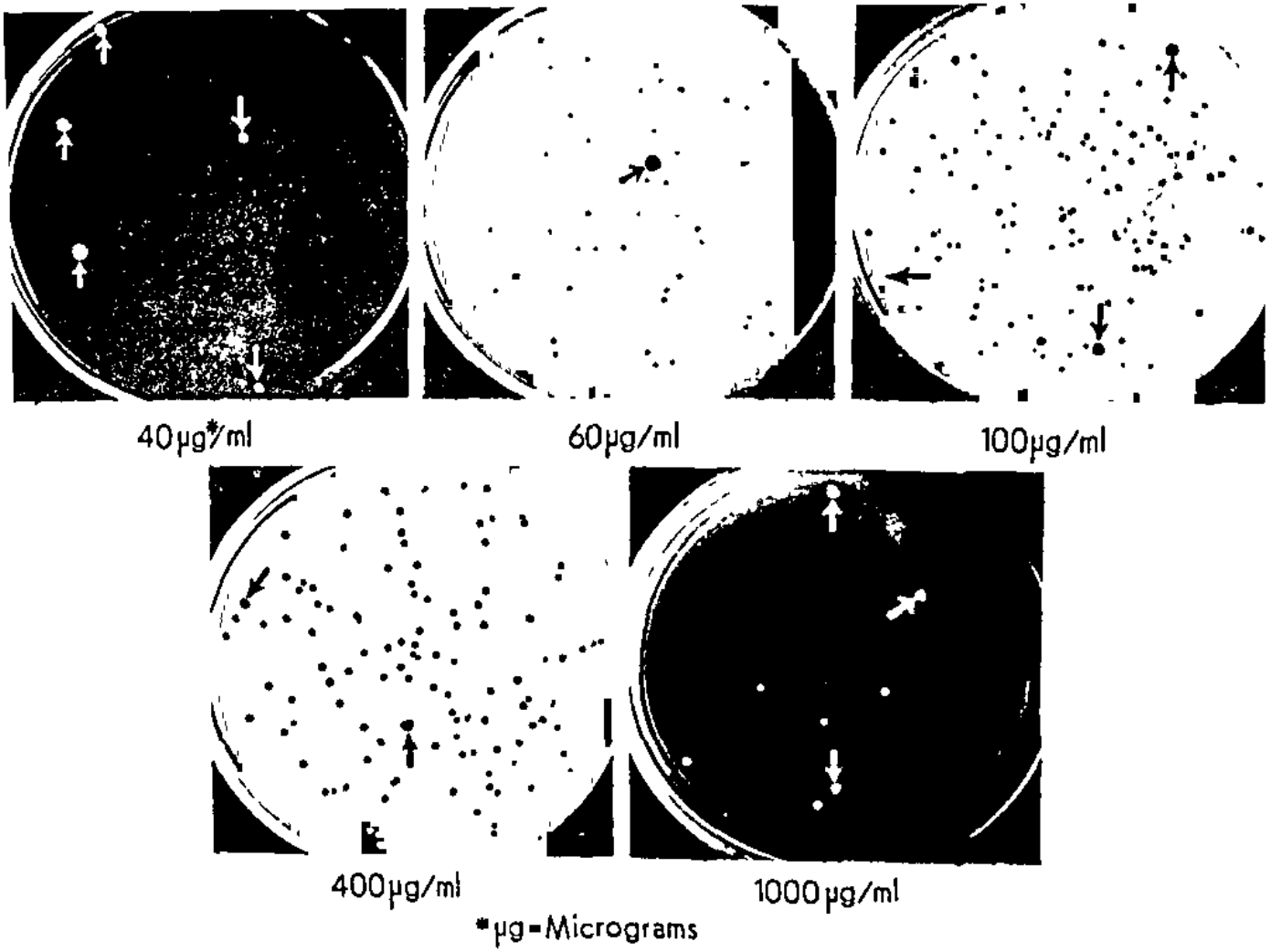

FIG 27 Streptomycin-resistant colonies are indicated by arrows, streptomycin-dependent colonies are unmarked.

Wyss, Stone and Clark (15a) have reported that irradiation of a culture of *S. aureus* with ultraviolet light or treatment with hydrogen peroxide or nitrogen mustard produced a significant increase in the incidence of variants resistant to 3.0 μg of streptomycin per milliliter.

From the observations described thus far the following facts may be summarized:

1. Bacteria may develop resistance to streptomycin (a) *in vivo*, that is, in clinical infections during the course of streptomycin therapy; or (b) *in vitro*, that is, during cultivation on artificial media containing streptomycin
2. The rate at which streptomycin resistance develops during serial cultivation on streptomycin media tends to be proportional to the number of bacteria transferred at each subcultivation.

   With small inocula such as are ordinarily used for serial transfer in broth containing increasing concentrations of streptomycin, resistance rises slowly in a step-wise fashion. When somewhat larger inocula are used, the resistance tends to rise more rapidly and may suddenly jump to a very high level. When sufficiently large inocula are used, resistance of a very high order may be developed by a single cultivation of a susceptible strain on high concentrations of the drug.
3. Since the presence of streptomycin in media acts to inhibit the reproduction of all microorganisms sensitive to the concentration used and permits the growth of only those which are resistant to that concentration, it is evident that the bacterial population of a "susceptible" strain is not homogeneous with respect to its susceptibility but contains a very small proportion of organisms that possess varying degrees of resistance. The highest degree of resistance is least common, moderate resistance more frequent, and slight degrees most frequent within this small fraction of the population In other words, the degree of resistance tends to be inversely proportional to its frequency

   It may seem contradictory to describe the presence of resistant organisms in a susceptible strain unless one remembers that they constitute such a small fraction of the population that they are not likely to be included in a random sample
4. Resistant variants appear in a culture started from a single colony of susceptible organisms, presumably descendants of a susceptible individual and after their appearance produce resistant progeny.[1] Resistance, is, therefore, a heritable character arising by mutation, which is assumed by Demerec (16) to be an example of gene mutation.

   The differences in degree of resistance possessed by mutants are explained by Demerec on the supposition that any one of several genes is involved in the development of resistance and that they differ from one another in "potency." If mutation occurs in a gene of low potency, resistance will be increased to a

[1] This can be demonstrated by suspending a single colony of a susceptible microorganism, for example, meningococcus, in 0.1 ml of saline and inoculating a loopful on each of several concentrations of streptomycin and also on streptomycin-free agar If no growth occurs on concentrations above 5 μg/ml, the original colony may be considered to consist entirely of susceptible organisms As the population increases on streptomycin-free media, subcultivation from it on streptomycin agar will demonstrate the presence of increasing numbers of resistant variants.

all concentrations of streptomycin between 40 and 4,000 μg/ml, though the number varied from strain to strain. For most strains their frequency was estimated to be about 1 to $3/10^{10}$ microorganisms inoculated, though higher frequencies were occasionally encountered if many determinations were made on a single strain (11). These streptomycin-resistant colonies were assumed to develop from resistant mutants that were constantly arising in the original bacterial population.

Alexander and Leidy (12) examined the strains of *H. influenzae* from fourteen patients treated with streptomycin for meningitis. From three of four patients who failed to recover, microorganisms which grew luxuriantly in media containing 1,000 μg of streptomycin per milliliter were isolated after treatment. A careful *in vitro* study of all the strains obtained before treatment showed no correlation, however, between the subsequent clinical response to streptomycin therapy and the tendency of the strains to develop resistance, for all the strains were able to give rise to streptomycin-resistant variants.

The incidence of organisms resistant to 1,000 μg/ml in ten of the strains varied from 1 in 1.1 billion to 1 in 13.8 billion, but these frequencies were distributed in random fashion, irrespective of the ultimate effect of streptomycin therapy on the infections which they had produced.

From this study, the authors concluded that survival of organisms which can grow in high concentrations of streptomycin either within the human host or *in vitro* is influenced more by the size of the bacterial population than by any other known factor.

Using a modification of Demerec's method, Alexander and Leidy (13) made careful systematic determinations of the frequency of resistant organisms in ten strains and found it to vary from 2.6×10^{-11}, to 7.0×10^{-11}, a variation which was not significant. These results confirmed their theory that resistant variants appeared in cultures of susceptible strains as a result of random mutation which was constantly occurring in the bacterial population.

Klein and Kimmelman (14) observed that most but not all strains of Shigellae developed resistance rapidly When 400 samples of 0.1 ml were transferred from a broth culture of *Sh dysenteriae*, it was found that 1 per cent of them contained variants which were resistant to 1,000 μg/ml. Klein (15) examined seven strains of bacteria, including *E. coli*, *Pr vulgaris*, and *S. aureus*, by testing 100 inocula of 0.4 ml each for their streptomycin resistance At least 1 per cent of the samples contained organisms resistant to high concentrations of streptomycin. It is interesting to note that a similar search for penicillin-resistant variants among a number of strains of *Staphylococcus* revealed none that possessed a high degree of resistance.

ing growth on high concentrations of streptomycin. Reversion to normal appearance occurred during subsequent cultivation on blood agar.

MICROSCOPIC APPEARANCE OF RESISTANT VARIANTS

Murray *et al.* (7) noted considerable pleomorphism in the gram-negative bacilli which had acquired moderate resistance during cultivation in streptomycin broth. Less pleomorphism was shown by the same strains grown on streptomycin agar.

Stubblefield (23) described a round cell variant of *E. coli* which was obtained from a single colony and which was resistant to streptomycin. After cultivation on streptomycin-free media, this variant became identical with the parent strain.

BIOCHEMICAL REACTIONS

Many authors have noted that streptomycin-resistant organisms grow more slowly than their parent strains, and several authors have observed a slower rate of sugar fermentation by resistant strains. Few instances of marked alteration in biochemical activity have been reported. Seligmann and Wassermann (21) found that two of seven strains of *Salmonella* lost their ability to produce gas, and one of these did not produce H_2S; the other and a third strain showed marked delay in H_2S production. Gezon and Cryst (22) observed reduction in streptokinase and streptolysin S production by some resistant strains of haemolytic streptococci.

VIRULENCE

Few observations have been reported on the virulence of microorganisms which have acquired resistance to streptomycin. Miller and Bohnhoff (8, 10) found that highly resistant meningococci were as virulent for mice as the parent strain from which they derived, and produced infections against which large doses of streptomycin afforded no protection. Seligmann and Wassermann (21), on the other hand, reported that all of seven strains of *Salmonella* lost their virulence for mice after they had become resistant to streptomycin. Gezon and Cryst (22) observed some reduction in mouse virulence of resistant beta haemolytic streptococci.

SENSITIVITY OF RESISTANT VARIANTS TO OTHER ANTIBACTERIAL AGENTS

Most investigators have found that strains which had acquired resistance to streptomycin retained their sensitivity to other antibiotics and to the sulfonamides. Klein and Kimmelman (24) found no evidence of cross resistance and showed that the combination of penicillin and/or

slight degree, but if it occurs in a highly potent gene, resistance of a high degree will result.

It is in this respect that the mutations involved in streptomycin resistance differ from those involved in the development of penicillin resistance. The latter, according to Demerec, are all of low "potency," an explanation which accounts for the gradual step-wise rise in penicillin resistance.

PERMANENCE OF STREPTOMYCIN RESISTANCE

Most highly resistant organisms have been found to retain this property during prolonged subcultivation on streptomycin-free media. Alexander and Leidy (13), for example, observed no change in one strain after 6 months of 24- or 48-hour transfers on streptomycin-free media. Miller and Bohnhoff (10) reported no loss in resistance in meningococci carried on streptomycin-free media for 1 year, nor did they observe any change during repeated passage in untreated mice. Knop (17) found no change in the level of resistance of several strains of gram-negative organisms after twenty-nine daily transfers in streptomycin-free media.

Murray, Wilcox, and Finland (18), on the other hand, found appreciable numbers of sensitive cells in two strains of gram-negative bacilli after twenty-eight and fifty-nine transfers respectively. Eleven other strains appeared to retain their resistance through 100 serial transfers in streptomycin-free broth. Chandler and Schoenbach (19) noted some loss of resistance in a strain of pneumococcus after 6 months' cultivation in streptomycin-free media.

Strains that have acquired only slight or moderate degrees of resistance have been found to lose it readily, usually at about the same rate at which the resistance was acquired (18, 20). These findings indicate that stability of resistant mutants varies from strain to strain and also suggest that back mutation occurs more frequently among mutants possessing low degrees of resistance These observations, however, demand confirmation by single-colony isolates.

MORPHOLOGY OF RESISTANT COLONIES

Most observers have failed to note any change in the appearance of streptomycin-resistant colonies, although several have observed that such colonies grow more slowly than the normal parent strains. Miller and Bohnhoff (9, 10) described a distinct yellowish color in the streptomycin-resistant variants of most of their strains of meningococcus. Loss of pigment formation as streptomycin resistance developed was observed by Seligmann and Wassermann (21) in two strains of *Ps. aeruginosa* and in two strains of *S aureus* by Graessle and Frost (20).

Gezon and Cryst (22) noted changes in colonial appearance and changes from beta to alpha or gamma haemolysis in haemolytic streptococci dur-

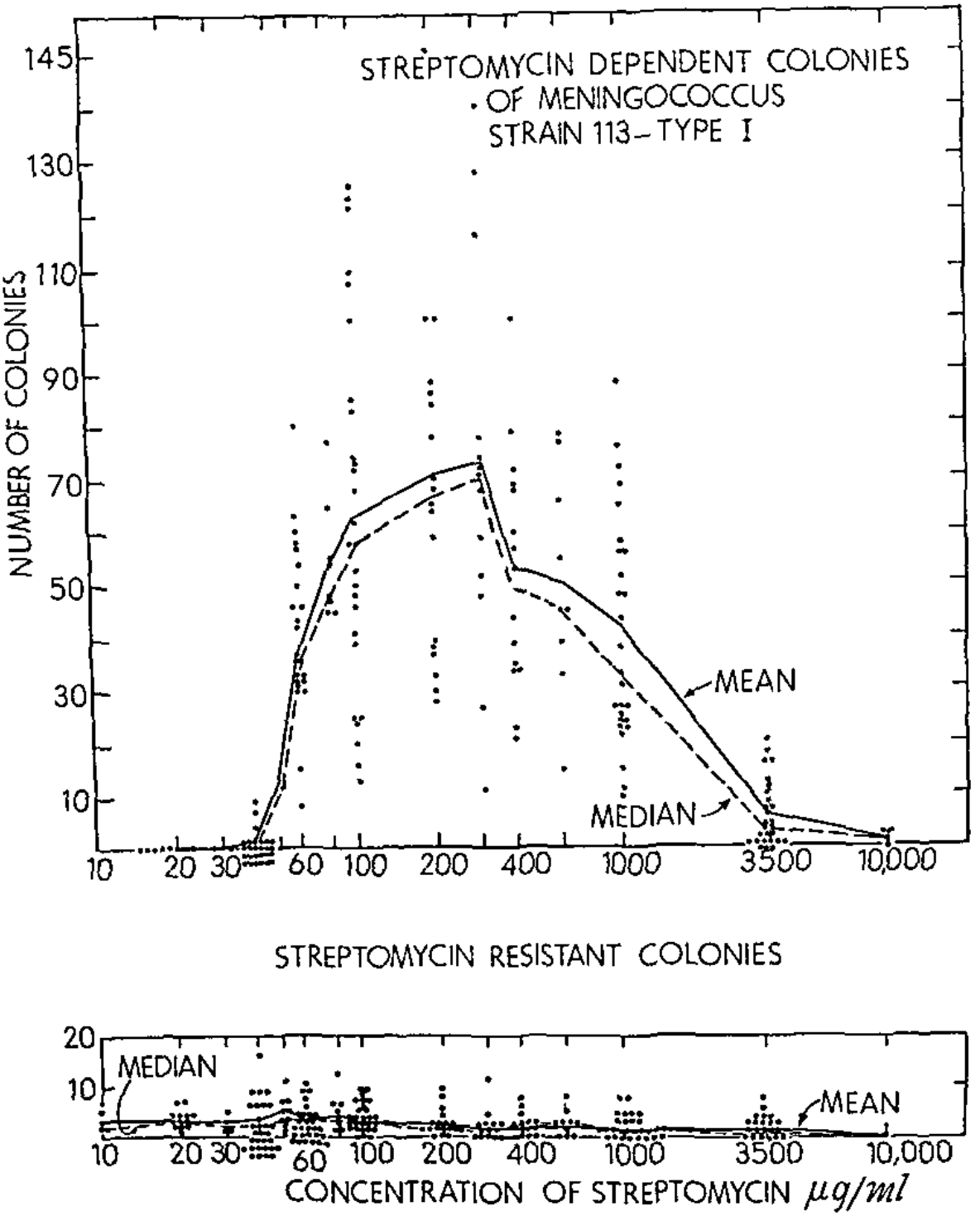

FIG. 29. Numbers of colonies of streptomycin-dependent and streptomycin-resistant variants developing from inocula of approximately 1.0 to 2.0 × 10^{10} susceptible meningococci on graded concentrations of streptomycin.

DEVELOPMENT OF STREPTOMYCIN DEPENDENCE

In their cultures of meningococcus on streptomycin-containing agar, Miller and Bohnhoff (9, 10) observed a second type of resistant colony in addition to the type A colonies already described. The second type, designated *type B*, also developed from susceptible strains during their

sulfadiazine with streptomycin was much more effective than either agent alone.

Klimek, Cavallito, and Bailey (25) observed slight to moderate increase in penicillin resistance in a strain of *S. aureus* after it had become highly resistant to streptomycin. Conversely, a high degree of penicillin resistance was accompanied by some increase in streptomycin resistance.

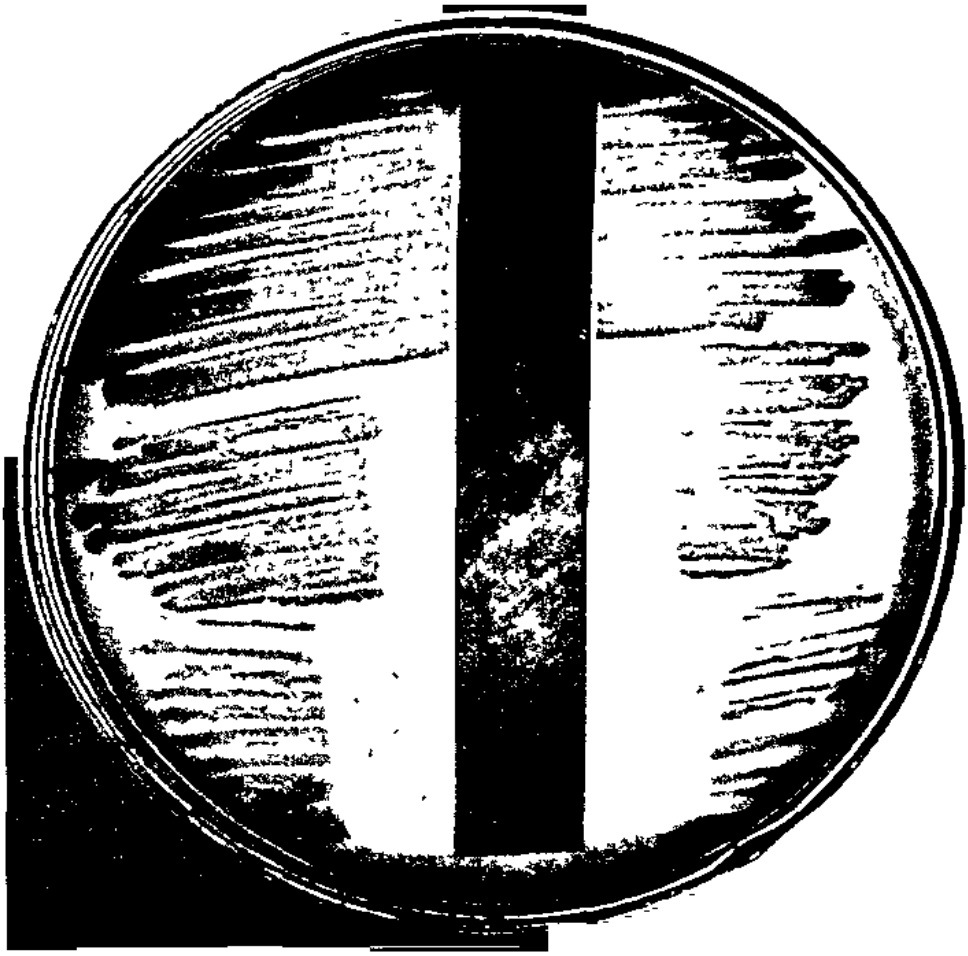

FIG. 28 Inhibition by penicillin (50 units per ml) in filter paper of top culture, penicillin-resistant meningococcus, middle, parent sensitive strain, bottom, streptomycin-resistant strain

Streptomycin resistance on the other hand, may be accompanied by increased sensitivity to penicillin, as is demonstrated in figure 28 which shows a wider zone of inhibition of streptomycin-resistant meningococcus than of the susceptible parent strain from which it was derived.

Graessle and Frost (20) found that two strains of *Staphylococcus* acquired some increase in streptomycin resistance as they became resistant to penicillin, whereas streptomycin-resistant strains remained sensitive to penicillin.

concentrations or from large dependent colonies on high concentrations. This fact indicates that the dependent variants were all genetically alike and that the larger size and slight pigmentation on the higher concentrations were due to a direct stimulating action of the streptomycin.

Streptomycin-dependent variants were obtained from all of eighteen strains of meningococcus and from a number of other bacteria, including, *A. aerogenes, E. coli, Proteus, Ps. acruginosa, Salmonella, and Staphylococcus* (26). Estimates of the mutation rate of dependent variants in one strain of meningococcus varied from two to fifteen per billion on optimal concentrations of streptomycin.

It is of interest that two sets of investigators mentioned the isolation of resistant variants which grew better on streptomycin-containing media. Some of the resistant variants of *H. influenzae* studied by Alexander and Leidy (13) were favored by the presence of streptomycin in the media.

TABLE 13

Results of inoculation of mice with streptomycin-dependent variant of meningococcus

NUMBER OF MENINGOCOCCI INOCULATED	STREPTOMYCIN TREATMENT	NUMBER OF MICE	RESULT	HEARTS' BLOOD CULTURES	
				On streptomycin media	On streptomycin-free media
10,000,000	No treatment	6	All survived		
100,000	10,000 μg (in 4 doses)	12	All died	Positive	Negative
10,000	during first 12 hours of infection	8	All died	Positive	Negative

Hall and Spink (3) noted that growth *in vitro* of the resistant strain of *Br abortus* isolated from the blood stream of a patient treated with streptomycin for bacterial endocarditis (referred to above) "was stimulated by the addition of sublethal concentrations of streptomycin."

Streptomycin requirement for experimental infection

Dependence on streptomycin can be demonstrated *in vivo* by inoculating mice with streptomycin-dependent meningococci and *E. coli* suspended in mucin (10). No infection results unless the mice are treated with streptomycin. Mice receiving adequate doses of the drug develop sepsis and die, and streptomycin-dependent organisms can be recovered from their hearts' blood in cultures on streptomycin-containing, but not on streptomycin-free, media. This fact demonstrates that the microorganisms retain their dependence on streptomycin during multiplication within the body of the infected animal host. The protocol of a typical experiment is presented in table 13.

initial exposure to streptomycin but grew more slowly. On concentrations between 60 and 100 μg, they differed from the type A colonies in that they were smaller, as can be seen in figure 27, and pearl-gray. On higher concentrations they grew to be larger and were less easily distinguishable from the type A colonies. Their most remarkable property was their dependence on streptomycin.

When equivalent inocula were planted on a series of plates containing graded concentrations of streptomycin, these colonies of streptomycin-dependent organisms always appeared in greatest numbers on concentrations between 100 and 400 μg/ml. The upper part of figure 29 shows the colony counts in thirty-five experiments in which a series of plates con-

TABLE 12

Number and appearance of colonies developing from small, equal inocula of pure culture of type B variant

STREPTOMYCIN	NUMBER OF COLONIES	DESCRIPTION
μg/ml		
0	0	
10	0	
40	3	Small, gray
60	8	Medium, gray
100	33	Medium to large, gray to slightly yellowish
200	35	Large, slightly yellowish
400	30	Large, yellowish
1,000	25	Large, yellowish
4,000	6	Small to medium, yellowish
10,000	0	

taining graded concentrations of streptomycin was inoculated with approximately equal quantities of a heavy suspension of a sensitive type I meningococcus.

Streptomycin-dependent variants would grow abundantly from small inocula on 100 to 400 μg/ml and would also grow from heavy inocula on concentrations as low as 5 μg/ml but not on lower concentrations. That concentrations between 100 and 400 μg/ml were optimal for the establishment of colonies is shown by table 12, which summarizes an experiment in which equivalent inocula of a pure culture of streptomycin-dependent meningococci were planted on a series of plates containing graded concentrations of streptomycin. It will be seen that once growth was initiated on streptomycin media, the colonies grew to a larger size on the higher concentrations Results identical with those shown in table 12 were obtained with subcultures from small dependent colonies growing on low

back mutation. A strain of *E. coli* with which they worked, however, was much less stable and tended to become resistant. Rake's experience (30), on the other hand, was different; his meningococci were less stable than *E. coli*.

Paine and Finland (29) found that dependent variants tended to give rise to streptomycin-sensitive organisms during prolonged growth in broth containing low concentrations of streptomycin. This suggests that back mutation was frequent among their strains. Purification of their dependent variants required several transfers in broth containing high concentrations of streptomycin in which growth had been most vigorous.

Microscopic appearance of dependent microorganisms

Smears made from dependent variants of meningococci growing on low concentrations of streptomycin showed these variants to be larger than normal meningococci (10). Organisms from colonies growing on higher concentrations were indistinguishable from the parent strain.

Dependent variants of the strains studied by Paine and Finland (29) showed marked pleomorphism but only when they were cultured in suboptimal concentrations of streptomycin.

Biochemical and immunological reactions

Streptomycin-dependent variants grow more slowly than their parent strains or streptomycin-resistant variants. Dependent variants of meningococcus show no other metabolic or biochemical differences. For example, they ferment dextrose and maltose, provided they are supplied with streptomycin. They retain their type specific agglutinability. No antigenic difference between them and their parent strains is detectable by agglutination or precipitin reactions with immune sera prepared from both.

Two strains of *Kl. pneumoniae* studied by Paine and Finland (29) failed to ferment dulcitol after becoming resistant or dependent. The dependent variant of one of the strains was inagglutinable and showed no capsular swelling in specific antiserum. The resistant variant showed capsular swelling and partial agglutination. Kushnick, Randles, Gray, and Birkeland (31) described the isolation of a streptomycin-dependent variant from a resistant strain of *B. subtilis* which would grow only under anaerobic conditions.

Sensitivity of dependent variants to other antibacterial agents

Paine and Finland (29) found only minor differences in the sensitivity of dependent variants of *S. aureus*, *E. coli*, *Proteus*, *Pseudomonas*, and *Kl. pneumoniae* to the action of penicillin, bacitracin, and polymyxin. Miller

As can be judged from the inocula used, the virulence of this dependent variant was much reduced.

Development of streptomycin dependence in vivo

The demonstration that streptomycin-dependent variants appear in bacterial cultures under experimental conditions raised the question of their possible occurrence *in vivo* during streptomycin therapy. Miller and Bohnhoff (26,27) searched for and found them in addition to large numbers of resistant organisms among the usual bacterial inhabitants of the throat and large bowel of mice and rabbits after 11 days of treatment with streptomycin. Streptomycin-dependent as well as streptomycin-resistant organisms were also recovered from the throats of patients during the course of streptomycin therapy.

Critical concentration of streptomycin

Paine and Finland (29) obtained streptomycin-dependent variants regularly from each of two strains of *S. aureus, Kl. pneumoniae, Pr. morganii,* and one of four strains of *E. coli* and one of three strains of *Pseudomonas.* They made the interesting observation (28) that the concentration of streptomycin in broth above which streptomycin-dependent variants multiplied was the same as or very close to the concentration above which growth of the sensitive parent strain was inhibited. In other words, there was a critical concentration of streptomycin, varying from strain to strain, above which the parent strain and below which its dependent variant were unable to grow.

The authors point out that although the exact mechanism of streptomycin action is not known, it may, in accordance with current theories, be assumed to act by blocking some essential metabolite or enzyme system. They point out further that it could scarcely be mere coincidence that the concentration of streptomycin which inhibits growth of a sensitive strain becomes necessary for the growth of a dependent variant of the same strain.

They raise the question whether the original essential metabolite may be replaced by some moiety of the streptomycin molecule which can be utilized in its place Resistant variants on the other hand grow independently of the presence or absence of streptomycin and may be thought of, therefore, as possessing some alternative mechanism or system that functions in the place of the one blocked by streptomycin in the sensitive strain.

Stability of dependent strains

Miller and Bohnhoff (9,10) found their strains of dependent meningococci to be markedly stable. The development of a single colony on streptomycin-free media occurred so rarely that it was regarded as the result of

back mutation. A strain of *E. coli* with which they worked, however, was much less stable and tended to become resistant. Rake's experience (30), on the other hand, was different; his meningococci were less stable than *E. coli*.

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Paine and Finland (29) found only minor differences in the sensitivity of dependent variants of *S aureus*, *E. coli*, *Proteus*, *Pseudomonas*, and *Kl. pneumoniae* to the action of penicillin, bacitracin, and polymyxin. Miller

As can be judged from the inocula used, the virulence of this dependent variant was much reduced.

Development of streptomycin dependence in vivo

The demonstration that streptomycin-dependent variants appear in bacterial cultures under experimental conditions raised the question of their possible occurrence *in vivo* during streptomycin therapy. Miller and Bohnhoff (26,27) searched for and found them in addition to large numbers of resistant organisms among the usual bacterial inhabitants of the throat and large bowel of mice and rabbits after 11 days of treatment with streptomycin. Streptomycin-dependent as well as streptomycin-resistant organisms were also recovered from the throats of patients during the course of streptomycin therapy.

Critical concentration of streptomycin

Paine and Finland (29) obtained streptomycin-dependent variants regularly from each of two strains of *S aureus*, *Kl. pneumoniae*, *Pr. morganii*, and one of four strains of *E coli* and one of three strains of *Pseudomonas* They made the interesting observation (28) that the concentration of streptomycin in broth above which streptomycin-dependent variants multiplied was the same as or very close to the concentration above which growth of the sensitive parent strain was inhibited. In other words, there was a critical concentration of streptomycin, varying from strain to strain, above which the parent strain and below which its dependent variant were unable to grow

The authors point out that although the exact mechanism of streptomycin action is not known, it may, in accordance with current theories, be assumed to act by blocking some essential metabolite or enzyme system. They point out further that it could scarcely be mere coincidence that the concentration of streptomycin which inhibits growth of a sensitive strain becomes necessary for the growth of a dependent variant of the same strain.

They raise the question whether the original essential metabolite may be replaced by some moiety of the streptomycin molecule which can be utilized in its place Resistant variants on the other hand grow independently of the presence or absence of streptomycin and may be thought of, therefore, as possessing some alternative mechanism or system that functions in the place of the one blocked by streptomycin in the sensitive strain.

Stability of dependent strains

Miller and Bohnhoff (9,10) found their strains of dependent meningococci to be markedly stable The development of a single colony on streptomycin-free media occurred so rarely that it was regarded as the result of

inhibitory to the reproduction of its parent strain is Emerson's mutant of *Neurospora* which requires sulfanilamide (33). Streptomycin-dependent mutation, however, occurs regularly in a number of bacterial species and in some at a rather high rate. In all of the eighteen strains of meningococcus examined by Miller and Bohnhoff, the mutation rate was higher for dependent than for resistant variants, and as can be seen by comparing the upper and lower charts in figure 29, the average frequency of the dependent variant on optimal concentrations was more than thirty-five times that of the resistant variant from the same strain.

Theoretical considerations

One is tempted to conclude that the streptomycin in the media does something more than permit the growth of mutants already present in the bacterial population; that is, that streptomycin induces this mutation. Such a conclusion, however, would involve a departure from conventional genetics which cannot be justified by any available experimental data. One seems obliged, therefore, to explain the occurrence of streptomycin-dependent variants in accordance with current genetic theory as resulting from random mutation which is constantly taking place in the population of a susceptible strain. On ordinary media such mutants must of necessity be nonviable because they occur in the absence of streptomycin.

Such a theory would become less plausible if this phenomenon should be found to occur with respect to a large number of antibacterial agents, because it would presuppose the appearance of more nonviable mutants than would be consistent with known facts about the rate of bacterial reproduction

The most helpful clue to the nature of the change accompanying streptomycin-dependent mutation is supplied by the observation of Paine and Finland that the concentration of streptomycin above which a dependent mutant is able to grow approximates that which completely inhibits the growth of the susceptible parent strain. The change must involve a vital process and would seem to be a relatively simple one. It can be most easily explained by assuming streptomycin to be an analog of a coenzyme in some reaction essential for growth. In susceptible cells, this reaction is blocked by streptomycin, but as a result of the mutation some minor change occurs which permits streptomycin to function as the coenzyme and thereafter to be itself essential for growth. This hypothesis is based on the studies of Fildes (34) and Woods (35) on the action of sulfonilamide.

The high concentration of streptomycin required for optimal growth of dependent organisms has raised the question whether it is streptomycin itself or some impurity which acts as the essential metabolite. Among

and Bohnhoff (10) noted the dependent variants of meningococcus to be as sensitive to penicillin as the normal parent strains from which they were derived.

The dependent variant of *M. ranae* isolated by Yegian and Budd (32) was found to be considerably more resistant to sulfathiazole than either the parent or the resistant strain. Whereas the sensitive and resistant strains were inhibited by 1 mg/100 of sulfathiazole, the dependent strain was able to grow in 100 mg/100.

Replacement of streptomycin

Miller and Bohnhoff (11) as well as Rake (30) found streptomycin, mannosido, and dihydrostreptomycin to be effective in supporting growth of streptomycin-dependent meningococci and *E. coli*.

Miller and Bohnhoff (26) were unable to find any other substance that would substitute for streptomycin in supporting the growth of dependent meningococci, although they tried streptamine, streptidine, streptobiosamine and other degradation products of streptomycin, lipositol, inositol, and streptomycin that had been inactivated by hydroxylamine HCl and cysteine HCl.

Rake (30), however, found some activity in two preparations of lipositol and also in n-acetyl mannosidostreptomycin and streptobiosamine. The activity found in n-acetyl mannosidostreptomycin and streptobiosamine was thought to be due to contamination of these products with streptomycin residue

General considerations

Streptomycin-dependent variants have been isolated from a sufficient variety of bacteria to indicate that their appearance represents a common phenomenon among microorganisms. Failure to demonstrate them may well be due to inadequacy of a systematic search on optimal concentrations. Since many of them are indistinguishable in colonial appearance from resistant variants, they can be identified only by making duplicate subcultures from individual colonies on streptomycin-containing and streptomycin-free media. The colonial differences between the dependent and the resistant colonies of meningococcus described by Miller and Bohnhoff were most apparent on concentrations of streptomycin between 40 and 200 μg/ml.

The origin of the streptomycin-dependent variants raises some interesting questions concerning the genetics of bacteria and their biological processes Since dependent organisms retain their dependence during repeated subcultivation, this trait is inheritable and must, therefore, be regarded as the result of mutation.

An analogous instance of a mutant dependent on a substance that is

To determine whether streptomycin dependence in addition to or, instead of, resistance may have contributed to therapeutic failure, it would be necessary to culture equal inocula of an infected body fluid on streptomycin-containing and on streptomycin-free agar. The development of a greater number of colonies on streptomycin media would suggest the presence of dependent organisms in the inoculum Proof would be obtained by subculturing a number of colonies in duplicate on streptomycin-containing and on streptomycin-free agar to determine the dependence of these colonies on streptomycin.

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many preparations of streptomycin, however, some of which were almost chemically pure, the activity in supporting growth of dependent mutants has been proportional to the content of streptomycin. There is no experimental evidence, therefore, that any substance other than streptomycin itself is the essential metabolite for the dependent variants.

Comment

Streptomycin resistance and dependence are phenomena of interest to investigators in the fields of bacterial genetics and bacterial metabolism, as well as to those concerned with the mode of action of antibacterial agents. Both streptomycin-resistant and streptomycin-dependent organisms have been found to be useful in the identification of streptomycin-producing molds (36, 37, 38).

Additional data need to be accumulated on the genetics of mutants of low or intermediate degrees of resistance. Such studies can be more profitably carried out on young colonies growing on solid media than with broth culture because young colonies consist of populations that are much more nearly homogeneous.

Metabolic studies on both resistant and dependent variants should prove helpful in explaining the mode of action of streptomycin

The development of streptomycin resistance is of great importance to the clinician in his therapeutic application of the drug. It is a matter of greater concern than is penicillin resistance, because it develops more readily and to a higher degree, and also because the dosage of the drug is limited by its toxicity

Needless administration of streptomycin to patients can be avoided by repeated bacteriological examination for the detection of streptomycin-resistant organisms during the course of treatment. Cultures should be planted directly on media containing several concentrations of streptomycin within the range which might reasonably be attained in the infected body fluid that the drug is expected to sterilize This procedure will prevent a misleading result that may occur if sensitivity determinations are made on a culture that has first grown out in streptomycin-free broth, because streptomycin-resistant mutants may appear in the bacterial population during its growth

Although it has been shown that streptomycin-dependent bacteria may be isolated from patients and animals undergoing treatment with streptomycin, there have been no published reports of cases in which the development of streptomycin dependence has been shown to play a role in the failure of streptomycin therapy This possibility cannot be ruled out, however, because it is not customary to make cultures from patients on streptomycin-containing media

To determine whether streptomycin dependence in addition to or, instead of, resistance may have contributed to therapeutic failure, it would be necessary to culture equal inocula of an infected body fluid on streptomycin-containing and on streptomycin-free agar. The development of a greater number of colonies on streptomycin media would suggest the presence of dependent organisms in the inoculum Proof would be obtained by subculturing a number of colonies in duplicate on streptomycin-containing and on streptomycin-free agar to determine the dependence of these colonies on streptomycin.

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CHAPTER 11

RESISTANCE OF TUBERCLE BACILLI TO STREPTOMYCIN

An ideal antibacterial agent for a chronic, slow-healing disease, such as tuberculosis, should be highly bactericidal for the infecting microorganisms in concentrations below the toxic range of the drug for the host. If the agent is primarily bacteriostatic, however, with only limited ability to destroy the invaders, it must be given over a long period to check the disease. During this period in which further multiplication of bacilli is retarded, the natural defense mechanisms of the host are allowed to initiate processes of repair, isolation of the infected areas, and destruction of the pathogenic organisms.

It is generally agreed that in tuberculous infections, streptomycin is predominantly a suppressive or bacteriostatic agent, having little bactericidal power. During prolonged administration of the drug, it has been observed that a large percentage of patients yield tubercle bacilli that are resistant to the action of streptomycin in the test tube. Since it has now been conclusively demonstrated that animals infected with such resistant organisms do not respond well to the drug and that, likewise, patients harboring these strains are not benefited by further courses of the drug, it is obvious that this phenomenon of resistance is one of the major obstacles to the clinical use of streptomycin for tuberculosis.

In assessing the value of streptomycin in tuberculosis, and the significance of drug-resistant bacilli in patients, host resistance becomes a factor of paramount importance. Interruption of the reproductive cycle of streptomycin-sensitive tubercle bacilli probably reduces certain metabolic and breakdown products of the organisms which may cause necrosis of tissue. This diminution of toxic products manifests itself by an early decrease in the volume and the bacillary count of the sputum, a regression of temperature, and a feeling of general well-being of the patient, followed by an improvement in the x-ray picture. Despite these favorable signs, however, many living tubercle bacilli may still be embedded deep in the tissues surrounding the open areas that previously were discharging bacilli into the sputum.

These remaining organisms, regardless of their sensitivity to streptomycin, must be walled off or removed by the mechanisms of host immunity, in order to effect a lasting arrest of the disease. Lacking such host resistance, the gains achieved by streptomycin therapy will be only temporary, whether the bacilli are sensitive or resistant to the drug.

Methods of Determining Streptomycin Sensitivity

Before the emergence of streptomycin-resistant tubercle bacilli and other related phenomena are discussed, the methods employed to determine streptomycin sensitivity of tubercle bacilli will be reviewed briefly.

The *in vitro* sensitivity to streptomycin of tubercle bacilli may be tested in either liquid or solid media. By either method, a measured inoculum of the bacteria is planted in a series of tubes of medium containing graded amounts of the drug, as well as in a control tube without streptomycin.

Liquid media

The Tween-albumin medium of Dubos (1), or any other suitable synthetic medium, such as the modification of Proskauer and Beck's medium described by Youmans (2), with or without addition of serum, may be used. In the medium containing Tween, growth of the organisms appears as a diffuse turbidity, whereas in a medium without a dispersing agent, growth occurs in aggregates The formula for the Tween-albumin medium should contain 0.02 per cent of Tween 80 and 0.5 per cent of albumin, as it has been demonstrated that in the presence of higher concentrations of Tween 80 and less of albumin, the action of streptomycin may be enhanced on certain cultures (3) When this revised formula is used, there are no appreciable differences in the results obtained with the Tween-albumin medium and with the serum-synthetic medium (4)

To perform the test, the sputum, or other clinical material, is first cultured on a solid medium in the routine manner The resultant growth is transferred to the Tween-albumin medium with a platinum spade and incubated for about 7 to 10 days A specified amount, usually 0.1 ml, of this dispersed growth is inoculated into the series of tubes of liquid medium containing streptomycin An alternative method is to grind some of the growth obtained on the solid medium in a sterile mortar with phosphate buffer, and to inoculate a specified amount of this suspension. The tubes are incubated for 14 days, when the final readings are made The sensitivity is expressed as the lowest concentration of streptomycin which prevents growth.

Solid media

Any good solid medium for the growth of tubercle bacilli may be used. In the case of egg-yolk–potato media, which have to be inspissated after

addition of streptomycin, a certain amount of the drug will be destroyed or rendered unavailable by adsorption to the proteins in the medium. The amount of available streptomycin in the medium, however, may be determined accurately by chemical assay from a representative sample of tubes. In this manner, it has been determined that by adding sufficient streptomycin to the egg-yolk–potato–glycerin medium recommended by the Committee on Laboratory Procedures of the American Trudeau Society (5), to give concentrations of 10, 50, and 500μg/ml, the tubes, when ready for use, *contain 3 5, 15, and 200 μg/ml.* Loss of streptomycin during inspissation may be obviated by using an agar-base medium, such as Herrold's egg yolk agar (6,7), or the oleic acid agar of Dubos (1).

The most rapid method of estimating sensitivity is to plant the concentrated and digested sputum *directly* on a series of tubes of medium containing graded concentrations of the drug and on a control tube without streptomycin. With this method, the result may be obtained in 18 to 26 days in highly positive specimens. On the other hand, if only a few colonies appear on the control tube, no accurate comparison can be made between the control tube and those containing streptomycin, and the growth must be further tested as a pure culture in either liquid or solid medium.

Another procedure that may be followed is to transfer the bacilli obtained from the routine culture to Tween-albumin liquid medium, and to inoculate the surface of a series of tubes of solid medium containing streptomycin with the resultant growth.

By using these solid medium modifications, a direct comparison can be made between the amount of growth on the control tubes and the amount of growth occurring in a given concentration of streptomycin. Thus, the *relative numbers of sensitive and resistant organisms can be estimated.*

Streptomycin Sensitivity of Various Strains of Tubercle Bacilli

The sensitivity to streptomycin of human and bovine strains of tubercle bacilli is remarkably constant. The effect of the drug on the virulent human strain, H37Rv, is demonstrated in figure 30, which shows the turbidity in Tween-albumin liquid medium plotted against days of incubation. Streptomycin in a concentration of 0.4 μg/ml is sufficient to inhibit growth completely for 21 days.

Youmans and Karlson (2) reported that of 131 human strains tested in the serum-synthetic medium, 118 were sensitive to 1.5 μg/ml or less, nine to 3 12, three to 0.25, and one required 12.5 for inhibition. Middlebrook and Yegian (8), using Tween-albumin medium, found that all of ninety human strains were sensitive to 1.0 μg/ml or less. In the Trudeau Laboratory (4), where the Tween-albumin medium was also used, it was observed

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μg/ml after about six transfers in Tween-albumin medium at 10-day intervals.

Tubercle bacilli may also be made resistant by growing the organisms on the surface of synthetic liquid media and serially transferring the pellicle of growth to fresh media containing increased concentrations of streptomycin. The rate at which drug resistance develops, however, is much slower than with the subsurface method of growing the organisms, as it requires many months to produce even a moderately resistant strain (4).

Williston and Youmans (10) were able to increase markedly the resistance of fourteen out of eighteen strains of human, avian, and bovine tubercle bacilli *in vitro*, three strains, however, showed only a fourfold increase in resistance, and one showed only a twofold increase. Twenty human strains, as well as the bovine strain, Ravenel, and the avian strain, Kirchberg, were tested at the Trudeau Laboratory (4), and all could be made highly resistant

Development of Resistant Tubercle Bacilli Experimentally in Vivo

In contrast to the rapidity and frequency with which streptomycin-resistant strains of tubercle bacilli appear in patients treated with the drug, tuberculous guinea pigs yield drug-resistant strains rarely, and then only when treatment has been given for many months. Feldman and his associates (11,12) have reported the finding of streptomycin-resistant tubercle bacilli in a total of eight guinea pigs, of which five had been treated with the drug for 215 days after intravenous infection and three for 146 days after subcutaneous infection. In October 1946, Feldman (13) reported, "The development by tubercle bacilli of resistance to streptomycin in guinea pigs treated with this drug has not been observed up to the present time in my laboratory." This experience was based upon the findings in about 175 treated pigs. Of this number, however, approximately 140 had been treated for 60 days or less.

In researches at the Trudeau Laboratory (14,15), no increase in resistance to streptomycin of the infecting organisms was found in eighty-six guinea pigs treated with the drug for 40 to 72 days. Also, the tubercle bacilli from five pigs treated for 125 days retained their initial sensitivity to streptomycin Resistant organisms were obtained from two pigs (4), one treated for 360 days and the other for 445 days. These animals had been infected intracerebrally with sensitive organisms. In one animal, however, cultures from the brain were drug sensitive, whereas the cultures from the lungs, liver, spleen, and inguinal lymph node were resistant. In the other guinea pig, tubercle bacilli from the inguinal node and the spleen were resistant, those from the lungs were sensitive, and the brain yielded a negative culture. From these findings, it might be postulated

that all of about 250 strains of tubercle bacilli isolated from patients prior to streptomycin therapy were inhibited by 1.0 μg/ml or less. The Veterans Administration (9) reported on 2,000 patients whose organisms were tested; approximately 97 per cent were sensitive to 10 μg/ml or less, and virtually all of these were inhibited by 1.0 μg.

Bovine tubercle bacilli have about the same sensitivity as the human bacilli. Of sixteen bovine strains tested by Youmans and Karlson (2), fifteen were inhibited by 1.5 μg/ml or less, and one was sensitive to 3 12. Avian strains, however, have been found to be approximately ten times

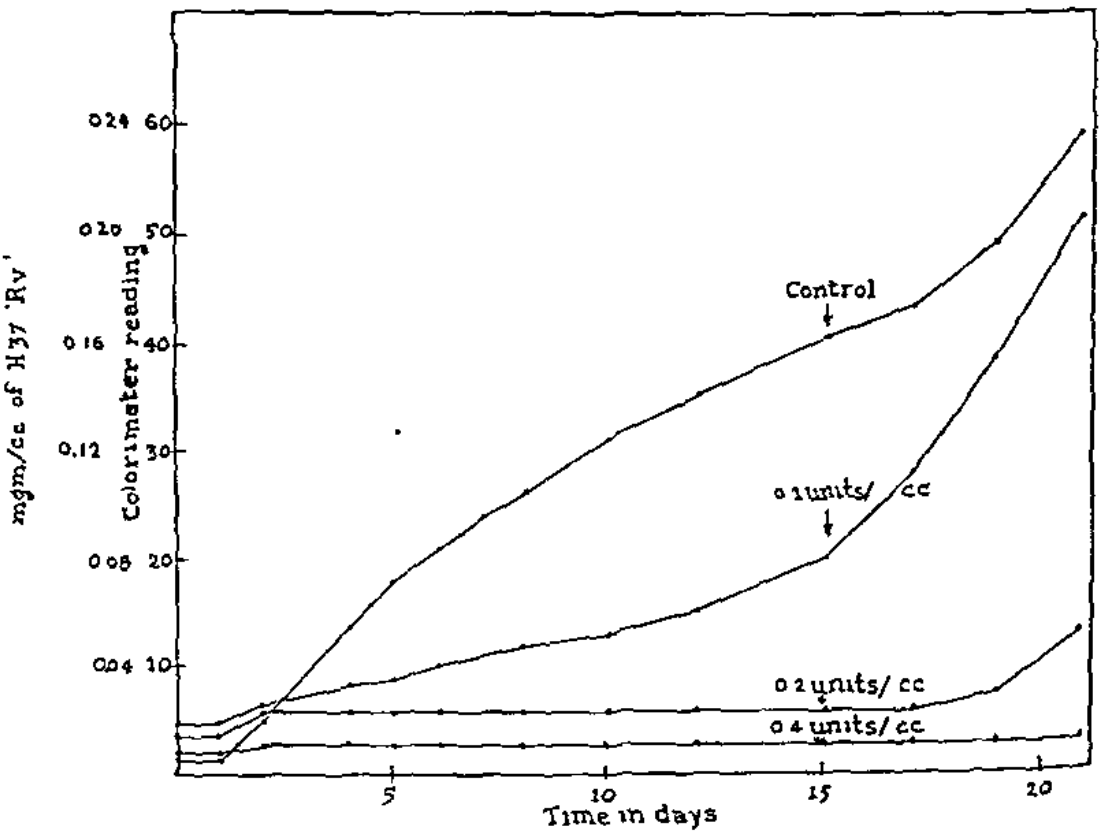


FIG 30 Effect of streptomycin upon the growth of H37Rv in Dubos medium (33)

more resistant Four out of fourteen avian strains tested by Youmans and Karlson (2) required 25 to 50 μg ml for inhibition, and only three were sensitive to 1 5 or less

DEVELOPMENT OF STREPTOMYCIN-RESISTANT TUBERCLE BACILLI IN VITRO

Tubercle bacilli, like other types of microorganisms, can be made highly resistant to the action of streptomycin in the test tube. This is easily accomplished by growing large inocula under the surface of enriched liquid media containing gradually increased concentrations of the drug. By this method, one can produce a strain of H37Rv that will grow readily in 1,000

The influence of daily dosage on development of resistant organisms is illustrated by figure 31. Three regimens are compared: 1.8 to 2.0 gm daily, 1.0 gm daily, and 0.5 gm daily for 120 days. Obviously, there is no significant difference in the rate of development or final percentage of resistant cultures. Even with the employment of maximum tolerated dosage of 3.0 gm daily for 120 days, more than 80 per cent of patients still excreting tubercle bacilli yielded drug-resistant cultures (18).

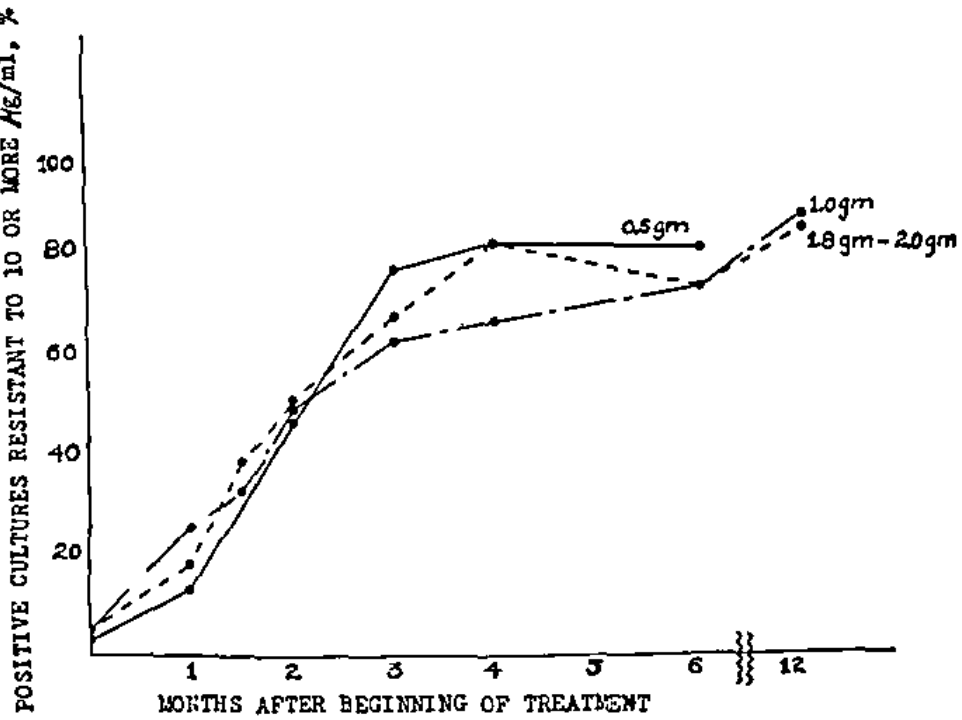


FIG. 31. tum remain data presen tion, held in ein for 120 days with 1.8 to 2.0 gm daily, 611 treated for 120 days with 1.0 gm daily, ninety-six treated for 120 days with 0.5 gm daily

The degree of resistance manifested by cultures varies widely. In some patients a culture sensitive to 0.5 μg/ml may be replaced 7 days later by a culture resistant to the action of 1,000 μg/ml (4). In other patients the organisms may show gradually increasing resistance from week to week. This increasing resistance may continue until the culture becomes resistant to 1,000 μg, but it may stop at any point before it reaches this high degree. Thus, there are patients whose cultures become resistant to 2.5 μg and sensitive to 5.0 μg, or resistant to 60 and sensitive to 125 μg/ml, and remain at that same level of sensitivity despite the continuation of treatment (see table 18).

Usually, if a patient finishes his course of streptomycin treatment with sensitive organisms, his cultures will continue to manifest susceptibility

that the resistant organisms were introduced into these animals by way of the common syringes and streptomycin vials, since guinea pigs infected with drug-resistant tubercle bacilli were being treated at that time from the same vials of streptomycin. In view of this possibility, rigid techniques must be employed to eliminate cross-infection; otherwise the results of such determinations of resistance may be misleading.

Youmans has studied the development of drug-resistant strains in infected mice treated with streptomycin. After treatment of seventeen mice for 35 days (16), the tubercle bacilli obtained from all the animals still retained their initial sensitivity In a later experiment (17), it was found that, as the daily dosage of streptomycin was increased, and the survival time lengthened, the percentage of mice yielding resistant strains increased. Thus, with the administration of 375 μg per day, which permitted an average survival time of 40 days, 22 per cent of the mice had resistant strains at death or sacrifice, with 1,500 μg per day, giving an average survival time of 132 days, 89 per cent had resistant strains.

It would appear, therefore, that the same strains of tubercle bacilli become resistant to streptomycin much more readily in the body of the mouse than in the body of the guinea pig. This may explain, at least in part, the ability of streptomycin to suppress to a greater degree the experimental disease in the guinea pig than in the mouse It also poses some very interesting and fundamental questions as to how and why tubercle bacilli become drug resistant *in vitro*.

Streptomycin-resistant Organisms from Patients

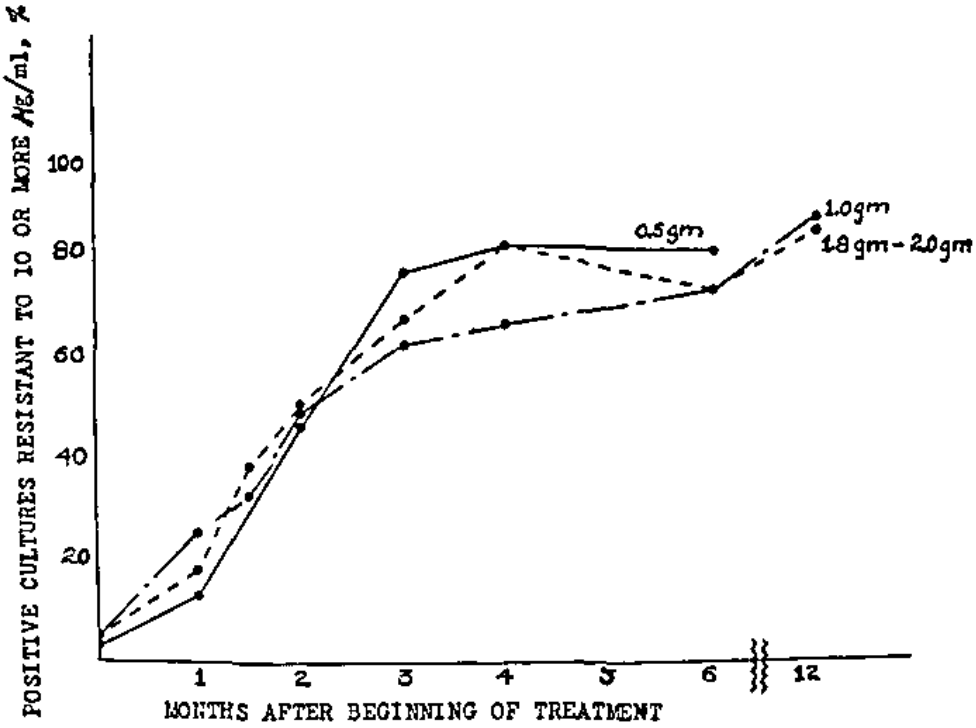
Although virtually all strains of tubercle bacilli recently isolated from patients prior to antibiotic therapy are sensitive to streptomycin, this sensitivity is frequently lost during treatment with the drug. The most important factor in determining the emergence of drug-fast organisms is the duration of treatment.

Figure 31 and table 14 show that the percentage of those patients who remain positive for tubercle bacilli and yield cultures resistant to 10 or more μg/ml increases steadily as treatment is continued.

The initial appearance of drug-resistant cultures may occur at any time during therapy, but the most frequent period is from the 4th to the 8th week Table 15 shows the time of initial appearance of drug-resistance in 31 patients with pulmonary tuberculosis treated with streptomycin. More than half of these resistant cultures first appeared during the 4th to the 8th week of therapy, three patients showed no evidence of resistant organisms until the 12th to 16th week

The earliest appearance of a drug-resistant culture that we know of occurred on the 29th day of therapy

The influence of daily dosage on development of resistant organisms is illustrated by figure 31. Three regimens are compared: 1.8 to 2.0 gm daily, 1.0 gm daily, and 0.5 gm daily for 120 days. Obviously, there is no significant difference in the rate of development or final percentage of resistant cultures. Even with the employment of maximum tolerated dosage of 3.0 gm daily for 120 days, more than 80 per cent of patients still excreting tubercle bacilli yielded drug-resistant cultures (18).



The degree of resistance manifested by cultures varies widely. In some patients a culture sensitive to 0.5 μg/ml may be replaced 7 days later by a culture resistant to the action of 1,000 μg/ml (4). In other patients the organisms may show gradually increasing resistance from week to week This increasing resistance may continue until the culture becomes resistant to 1,000 μg, but it may stop at any point before it reaches this high degree. Thus, there are patients whose cultures become resistant to 2.5 μg and sensitive to 5 0 μg, or resistant to 60 and sensitive to 125 μg/ml, and remain at that same level of sensitivity despite the continuation of treatment (see table 18).

Usually, if a patient finishes his course of streptomycin treatment with sensitive organisms, his cultures will continue to manifest susceptibility

TABLE 14

Range of sensitivity to streptomycin of tubercle bacilli isolated from patients under treatment with 1 0 gm of streptomycin daily for 4 months (120 days) (31)

MG OF STREPTOMYCIN PER ML OF MEDIA NECESSARY TO INHIBIT GROWTH	BEFORE TREATMENT		AFTER 2 WEEKS' TREATMENT		AFTER 4 WEEKS' TREATMENT		AFTER 6 WEEKS' TREATMENT		AFTER 8 WEEKS' TREATMENT		AFTER 10 WEEKS' TREATMENT		AFTER 12 WEEKS' TREATMENT		AFTER 14 WEEKS' TREATMENT		AFTER 16 WEEKS' TREATMENT		AFTER 18 WEEKS' TREATMENT	
	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures	Number	Per cent of positive cultures
0 5 or less	12	91	42	91	23	62	7	21	5	14 5	2	7	2	6 5	1	3.5	0	0	0	0
0 6 to 1 0	3	6	2	4	8	22	6	17 5	5	14 5	3	10	2	6 5	1	3 5	1	3.5	1	3
2 0 to 5 0	0	0	1	2	5	13 5	11	32	6	17 5	5	16 5	4	13	5	16.5	5	16.5	4	13
10 to 31	0	0	0	0	1	2 5	6	17 5	7	21 0	5	16.5	4	13	3	10	2	7	1	3
62 to 125	0	0	0	0	0	0	2	6	3	9	5	16 5	6	19	5	16 5	4	13	5	16
250 to 500	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	3.5	2	7	2	7
Over 1,000	0	0	0	0	0	0	2	6	8	23 5	10	33 5	12	39	14	46 5	16	53	18	58
Tot 1 0 or less	45	100	42	91	31	84	13	38 5	10	29	5	17	4	13	2	7	1	3.5	1	3
Tot 5 0 or less	45	100	45	100	36	97 5	24	70 5	16	46 5	10	33.5	8	26	7	23.5	6	20	5	16
Tot 10 0 or more	0	0	0	0	1	2.5	10	29 5	18	53 5	20	66 5	23	74	23	76.5	24	80	26	84
Total	45		45		37		31		34		30		31		30		30		31	

to the drug. In rare instances, however, drug-resistant cultures have been obtained from such patients some time after the last dose of streptomycin has been given (see table 18). It has also been observed that patients who yielded negative cultures during treatment and for some time afterward have shown drug-resistant organisms at a later date when their cultures again became positive (4,19).

The sensitivity of tubercle bacilli obtained from one part of the body does not necessarily reflect the state of sensitivity of the organisms in other parts of the body. For example, in one patient (4) bacilli from the sputum are consistently resistant to streptomycin, whereas cultures from the urine made at the same time are sensitive. In the study of autopsy material, cultures from various sites in the body usually exhibit approximately the

TABLE 15

Duration of therapy at the time when each of 31 patients first demonstrated drug-resistant organisms (4)

DURATION OF TREATMENT	NUMBER OF PATIENTS	NUMBER SHOWING INITIAL APPEARANCE OF DRUG-RESISTANT CULTURES
weeks		
0– 2nd	96	0
2nd– 4th	96	6
4th– 6th	96	7
6th– 8th	82	9
8th–12th	70	6
12th–16th	59	3
Total		31

same sensitivity, but occasionally the bacilli from separate areas will show marked differences in susceptibility to the drug.

To explain these inconsistencies, one must assume that there is either a variation in the amount of contact between the bacilli and the drug (as in the poor penetration of streptomycin into the centers of large, avascular, caseous areas), or that the tissue itself, in which the bacilli are embedded, has some influence on the development of resistance.

Response to Streptomycin Therapy of Animals Infected with Resistant Organisms

The rapidity with which tubercle bacilli acquire drug resistance, as demonstrated by the *in vitro* test, was established in the early researches with streptomycin. This, of course, brought up the question of whether *in vitro* resistance corresponded with *in vivo* resistance and led to the inves-

tigation of the effect of streptomycin in tuberculosis produced by resistant cultures in experimental animals. It was uniformly discovered that in guinea pigs and in mice, infections produced by highly resistant microorganisms, whether from streptomycin-treated patients or from laboratory strains, did not respond to treatment with the drug (16,12,15). One group of investigators (15) reported earlier deaths in the streptomycin-treated animals than among the untreated controls infected with two different drug-resistant strains.

The effect of streptomycin on tuberculous disease produced in guinea pigs by cultures exhibiting various degrees of drug resistance has also been investigated (15).

The results (table 16) indicate that, in general, those cultures which proved to be resistant to 15 or more μg/ml, as tested in the Tween-albumin liquid medium, produced nonresponsive infections, whereas those sensitive to 2.5 or less μg/ml usually produced infections amenable to the drug. There were, however, some inconsistencies in the response of the guinea pigs infected with a few cultures of borderline resistance.

An interesting sidelight to this investigation was the finding that the strains of tubercle bacilli used to infect these animals showed no appreciable increase in resistance when recovered at the end of the experiment from the treated animals and tested in liquid and in solid media. One might have expected that strains which already possessed some degree of resistance would show an increase in resistance while residing in the streptomycin-treated pigs.

The data from the studies mentioned (12, 15, 16), as well as some unpublished observations (4), indicate that streptomycin-resistant strains of *tubercle bacilli have essentially the same virulence for guinea pigs and mice* as do their drug-sensitive parent strains.

Effect of Streptomycin on Early Tuberculosis in Guinea Pigs

Studies have been made on the effect of streptomycin on the rate of distribution of virulent tubercle bacilli, streptomycin-sensitive and streptomycin-resistant, from the site of inoculation in the inguinal region of guinea pigs (20, 21). It was found that the drug therapy, even though begun 48 hours prior to introduction of the organisms, had no effect on the immediate dissemination of the tubercle bacilli, either sensitive or resistant, throughout the body of the infected animal

The early pathogenesis of the disease in those animals infected with drug-sensitive tubercle bacilli and treated with streptomycin was as follows: Lesions were first noted in the iliac nodes after 10 days. Progression of the lesions was slow but definite up to 27 days, at which time caseation was present At 39 days, the next sacrifice period, only a few macrophages and

TABLE 16

Streptomycin treatment in guinea pigs infected with 21 cultures of tubercle bacilli exhibiting intermediate drug resistance in vitro (15)

PATIENT	DAYS OF TREATMENT	IN VITRO STREPTOMYCIN SENSITIVITY*	RESULTS OF TREATMENT OF GUINEA PIGS			SENSITIVITY OF RECOVERED TUBERCLE BACILLI								
			Ave degree of tuberculosis†		Response of treated pigs	Control					Treated			
			Control	Treated		Liquid*	Solid medium‡				Liquid	Solid medium		
							0	3 5	15	200		0	3 5	15	200
A. Z	21	0.5	14.6	1.3	Excellent						0.5	1	0	0	0
	28	2.5–5§	14.1	2 9	Good	1–2.5¶	1+	0	0	0	2.5–10	3	0	0	0
	42	5–10	15.0	3 8	Good	2 5	3+	0	0	0	10–30	25	25	15	0
E C.	7	0.5	13.1	1.0	Excellent	1 0	3+	0	0	0	Cultures negative				
	22	2 5–5	15.0	10 8	Poor	5 0	15	2	0	0	10	3	1+	0	0
	22	2 5–5	12 6	11 2	Poor	5 0	5	3	0	0	5–10	16	0	0	0
W. B.	23	0.5	14 0	2 8	Good	1.0	6	0	0	0	Cultures negative				
	84	2 5–5	14.1	13 0	None	2.5–10	4+	1+	0	0	5.0	12	1	0	0
R. H	48	0.5	14 0	2 3	Excellent	1.0	3+	0	0	0	0 5	2	0	0	0
R. G.	32	1–2.5	11.0	6 3	Fair						2 5	3	ct‖	0	0
A. F.	97	1–2.5	14.5	10 0	Poor	2.5	1+	0	0	0	2 5	5	0	0	0
D. F	120	2 5	14.5	6.2	Fair	1–2 5	2+	0	0	0	2 5	3	ct	0	0
M. K	31	2 5	13 6	1 9	Excellent	0.5–1	1+	0	0	0	Cultures negative				
T. C	42	2.5–5	15 5	10.7	Poor	1 0	1	0	0	0	10	25	7	0	0
G. D	37	5 0	14.5	9 8	Poor	2 5–5	3+	1+	0	0	5 0	2+	1+	0	0
E. L.	47	10	12.6	1 5	Excellent	0 5–1	30	0	0	0	Cultures negative				
M. P.	117	10–15	14.5	10 5	Poor	10	ct	1+	0	0	10	15	1	0	0
E. B.	45	15–30	14.5	11 6	Poor						60–125	1+	1+	30	0
N. M	68	30–60	13.6	13	None	30–60	3+	2+	10	0	60–125	3+	2+	1+	0
L. L.	103	125	15.4	12 5	Poor	125– 250	2+	2+	30	0	125	2+	1+	10	0
J T.	120	250	12 4	13 2	None	500–1000	2+	2+	2+	1	1000	3+	2+	2+	0

* Lowest concentration of streptomycin inhibiting growth for 14 days in Tween-albumin medium Concentrations used were: 0 (control), 0 5, 1 0, 2 5, 5 0, 10, 15, 30, 60, 125, 250, 500, and 1,000 μg/ml.

† Based on a maximum of 16 for each animal (4 each for the lungs, liver, spleen, and lymph nodes).

‡ Streptomycin was incorporated into the modified Committee Medium in concentrations of 10, 50, and 500 μg/ml After sterilization and inspissation of the solid medium, the actual concentrations of available streptomycin by chemical analysis were 3 5, 15 and 200 μg/ml. The animal tissues, after digestion and concentration, were planted on a series of these tubes containing streptomycin, as well as on two tubes of the plain medium. Growth is expressed in terms of 1 to 4+. If fewer than 50 colonies appeared, the actual number of colonies is recorded in the table. An average value for the positive cultures in each group is given.

§ When two values appear, they indicate the range of results on repeated tests of the same culture.

¶ Where two values appear, they indicate the range of sensitivities for all the positive cultures obtained from the group of animals

‖ Culture contaminated.

tigation of the effect of streptomycin in tuberculosis produced by resistant cultures in experimental animals. It was uniformly discovered that in guinea pigs and in mice, infections produced by highly resistant microorganisms, whether from streptomycin-treated patients or from laboratory strains, did not respond to treatment with the drug (16,12,15). One group of investigators (15) reported earlier deaths in the streptomycin-treated animals than among the *untreated* controls infected with two different drug-resistant strains.

The effect of streptomycin on tuberculous disease produced in guinea pigs by cultures exhibiting various degrees of drug resistance has also been investigated (15).

The results (table 16) indicate that, in general, those cultures which proved to be resistant to 15 or more μg/ml, as tested in the Tween-albumin liquid medium, produced nonresponsive infections, whereas those sensitive to 2.5 or less μg/ml usually produced infections amenable to the drug. There were, however, some inconsistencies in the response of the guinea pigs infected with a few cultures of borderline resistance.

[illegible] in resistance when recovered at the end of the experiment from the treated animals and tested in liquid and in solid media. One might have expected that strains which already possessed some degree of resistance would show an increase in resistance while residing in the streptomycin-treated pigs.

The data from the studies mentioned (12, 15, 16), as well as some unpublished observations (4), indicate that streptomycin-resistant strains of tubercle bacilli have essentially the same virulence for guinea pigs and mice as do their drug-sensitive parent strains.

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The early pathogenesis of the disease in those animals infected with drug-sensitive tubercle bacilli and treated with streptomycin was as follows: Lesions were first noted in the iliac nodes after 10 days. Progression of the lesions was slow but definite up to 27 days, at which time caseation was present. At 39 days, the next sacrifice period, only a few macrophages and

TABLE 17—*Continued*

PATIENT	DATE	MONTHS IN THE DEEP FREEZE	GROWTH ON SOLID MEDIUM	GROWTH IN TWEEN ALBUMIN LIQUID MEDIUM	STREPTOMYCIN SENSITIVITY*	ORIGINAL STREPTOMYCIN SENSITIVITY*
Y.S.	Sept. 16, 1946	22	Fair	Good	0–1.0	1000 (14)
	Oct. 18, 1946	21	Excellent	Excellent	1000 (5)	1000 (11)
	Nov. 20, 1946	20	Excellent	Excellent	1000 (4)	1000 (5)
	Dec. 11, 1946	19	Good	Good	1000 (4)	1000 (4)
	Jan 17, 1947	18	Good	Good	1000 (6)	1000 (7)

* Figures in parentheses are the days on which growth was first observed in the tube containing 1,000 μg/ml of streptomycin.

a small area of scar tissue were present. In the spleen, foci of macrophages were observed in the malpighian bodies at 21 days, but not before or after this period. At 27 days, small groups of macrophages and a few epithelioid tubercles were observed in several of the lymphoid foci in the lungs. At 39 days, both these and the lymphoid foci were absent, and the lungs appeared to be normal. It is apparent, then, that despite streptomycin therapy, the tuberculous lesions progressed for about 27 days, then regressed, and at 39 days the only lesions observed were a few macrophages and a scar in the iliac node. In marked contrast, the untreated controls showed rapid, continuous progression of the lesions in the lymph nodes, spleen, liver, and lungs.

In the animals that were infected with streptomycin-resistant organisms, lesions developed in the various organs at the same intervals after infection and progressed at similar rates in the treated as in the control animals. In many instances, there appeared to be fewer Langhans' cells associated with the lesions in the treated animals than in the controls, but no gross and no other histological distinctions could be made between the two groups.

It would appear that in the guinea pigs infected with streptomycin-sensitive microorganisms and treated with streptomycin, it was not until the animals had acquired a skin sensitivity to tuberculin that regression in the lesions took place It is known that tuberculin hypersensitivity and acquired host resistance appear almost simultaneously (22, 23) It is assumed, therefore, that some host resistance developed at this time. Thus, the cessation of progression and the appearance of a regressive tendency of the lesions in the treated animals coincided with the development of acquired host resistance.

Permanence of Streptomycin Resistance

Streptomycin resistance of tubercle bacilli, once acquired, is usually a relatively permanent characteristic In tubercle bacilli subcultured at frequent intervals on solid or liquid medium, and in cultures kept in the deep freeze, resistance has been maintained for 2 years

TABLE 17

Results of viability and streptomycin sensitivity of forty originally streptomycin-resistant cultures taken from the deep freeze (4)

PATIENT	DATE	MONTHS IN THE DEEP FREEZE	GROWTH ON SOLID MEDIUM	GROWTH IN TWEEN-ALBUMIN LIQUID MEDIUM	STREPTOMYCIN SENSITIVITY*	ORIGINAL STREPTOMYCIN SENSITIVITY*
F.B.	Oct. 17, 1946	21	Good	Poor	1000 (6)	1000 (14)
	Nov. 20, 1946	20	Excellent	Contaminated	1000 (12)	1000 (13)
	Dec. 12, 1946	19	Excellent	Fair	1000 (5)	1000 (8)
	Jan. 15, 1947	18	Excellent	Good	1000 (4)	1000 (8)
W.E	Oct. 18, 1946	21	Good	None	1000 (5)	30–60
	Nov 20, 1946	20	Excellent	Good	1000 (6)	1000 (7)
	Dec. 12, 1946	19	Excellent	Excellent	1000 (6)	1000 (6)
	Jan. 17, 1947	18	Excellent	None	1000 (4)	1000 (6)
	Feb 14, 1947	17	Good	Excellent	1000 (5)	1000 (7)
E G.	Feb 14, 1947	17	Good	Good	1000 (10)	1000 (12)
	Mar. 22, 1947	16	Good	Good	1000 (4)	1000 (8)
J G	Feb 14, 1947	17	Excellent	Excellent	1000 (5)	1000 (8)
J.L	Nov 20, 1946	20	Good	Poor	1000 (5)	1000 (5)
	Dec 12, 1946	19	None	None	—	1000 (6)
	Jan 17, 1947	18	Excellent	Excellent	1000 (4)	1000 (6)
	Feb 14, 1947	17	Excellent	Good	1000 (4)	1000 (6)
W.L	Feb 26, 1947	16½	Excellent	Excellent	1000 (12)	30–60
	Apr 2, 1947	15½	Excellent	Excellent	1000 (7)	1000 (6)
D M	Nov. 20, 1946	20	Good	None	250–1000	60–125
	Dec 12, 1946	19	Excellent	None	125– 250	60–125
	Jan. 17, 1947	18	Fair	None	125– 250	125–250
	Feb 14, 1947	17	Excellent	None	125– 250	60–125
	Mar 22, 1947	16	Excellent	Good	1000 (9)	60–125
O M	Dec 11, 1946	19	Fair	Good		1000 (7)
	Feb 14, 1947	17	Excellent	Good	1000 (6)	1000 (9)
	Mar. 19, 1947	16	Excellent	Excellent	1000 (7)	1000 (6)
	July 20, 1947					
	Post #1	12	Excellent	None	1000 (6)	1000 (6)
	Post #2	12	None	None	—	30–60
	Post #3	12	Excellent	None	1000 (4)	1000 (6)
	Post #4	12	Good	None	1000 (5)	1000 (6)
	Post #5	12	Good	Good	1000 (5)	1000 (5)
J T	Sept. 16, 1946	22	Good	Fair	2 5– 5	15– 30
	Dec 11, 1946	19	Excellent	Poor	250–500	60–125
	Jan 17, 1947	18	Excellent	Good	125–250	60–125
	Feb. 14, 1947	17	Excellent	Good	125–250	60–125

TABLE 18

Follow-up of streptomycin sensitivity of tubercle bacilli from patients after conclusion of streptomycin treatment (4, 19)

PATIENT	SENSITIVITY AFTER COMPLETION OF THERAPY	MONTHS AFTER TREATMENT	SENSITIVITY
	μg/ml		*μg/ml*
W E	>1000	20	>1000
J.K.	>1000	15½	>1000
J.G.	>1000	14	>1000
P.C.	>1000	11	>1000
G.H.	>1000	11	>1000
J.L.	>1000	10	>1000
Y.S.	>1000	1	>1000
		4	0.5
		8	0.5
E M	125	4	125 to 250
		9	250
		17*	1 125 2. 250 3. >1000 4 >1000
A.D.	15 to 60	15½	30
L.L. (ur)	125	13	250
C B.	125	12	125
T J	60 to 125	10	250
M C	60 to 125	6	30
		8	2 5
		10	0.5
M.M.	125 to 250	4	1.0
		7	0.5
F A	60 to 125	6½	250
		12½	15
		15*	2 5
J T.	30	6½	125
C.S	1.0	1	1.0
		2	60
		4	30
		5½	60
		8	60
		11½	5.0

* Indicates cultures obtained at autopsy.

Table 17 presents the results obtained when forty cultures of varying degrees of resistance, which had been kept in the deep freeze up to 22 months, were subcultured and tested for streptomycin sensitivity (4). Only two of the cultures failed to grow. With one exception, there was no appreciable loss of drug resistance during the stay in the deep freeze. The exception was the Y. S. Sept. 16, 1946 culture, which became completely sensitive again, after having once been resistant to 1,000 μg/ml. In addition to losing its resistance in the deep freeze, this culture was also noted to be sensitive again after about 3 months of transfer on solid medium.

Streptomycin resistance is also retained in vivo. A highly resistant H37 Rv culture lost none of its resistance after 8 months of repeated testicle passage in guinea pigs (24).

Table 18 presents the follow-up of drug resistance of tubercle bacilli obtained from patients after the conclusion of their treatment with streptomycin. The first group of six patients demonstrates the usual finding of highly resistant cultures 1 to 2 years later. Patient Y. S, however, illustrates the rare instance in which cultures may revert to sensitivity again after the patient has yielded organisms resistant to 1,000 μg/ml at one time.

The next group of five patients represents those whose cultures had acquired an intermediate degree of resistance, which remained relatively constant on repeated isolations for many months. Two of these patients continued to produce organisms of about the same resistance despite second courses of streptomycin The results of sensitivity tests of cultures from M C, M. M, and F. A. demonstrate that certain patients who yield cultures of intermediate resistance during therapy may produce strains of decreasing resistance as time goes on This has been observed with greater frequency in this group of patients than in those who yield tubercle bacilli of high resistance (more than 1,000 μg/ml) during treatment.

The last two patients illustrate the unusual occurrences of the appearance of resistant strains for the first time, and the production of strains of increased resistance, after streptomycin therapy had been discontinued.

Naturally Occurring Streptomycin-resistant Variants

It has been repeatedly demonstrated that a small number of tubercle bacilli in a given culture may naturally possess a relative resistance to the action of streptomycin (7, 25, 26, 27, 28), even though the culture as a whole is highly sensitive. Pyle (25) found some of these isolated resistant variants in the sputa from seven of eight patients Each of these seven produced a small number of colonies on plates of medium containing 5 and 10 μg of streptomycin per milliliter, but no colonies appeared in medium con-

TABLE 19

Naturally resistant colonies of tubercle bacilli on committee medium, repeated cultures on patients who never had contact with streptomycin; sputum planted directly on series of streptomycin tubes (4)

PATIENT NUMBER	NUMBER OF POSITIVE CULTURES	DEGREE OF POSITIVITY	NUMBER OF CULTURES SHOWING NO COLONIES IN S'MYCIN TUBES	NUMBER OF CULTURES SHOWING COLONIES IN S'MYCIN TUBES	DISTRIBUTION OF RESISTANT COLONIES	
					Number	Streptomycin concentration
						μg/cc
1	9	4+	7	2	6 1	3 5 3 5
2	10	4+	9	1	2	3 5
3	9	3+ to 4+	9	0		
4	10	4 4+ 1 3+ 3 2+ 1 28 col 1 3 col	10	0		
5	7	4+	7	0		
6	10	4+	3	7	6 30 3 1 1 2 3	3.5 3 5 3.5 3.5 3 5 3.5 3.5
7	9	4+	1	8	4 4 2 1 3 2 7 9 2	3.5 3.5 3 5 15 3.5 3 5 3.5 3.5 3 5
8	10	3+ to 4+	10	0		
9	10	3+ to 4+	8	2	1 1	3.5 3 5
10	10	4+	8	2	1 3	3.5 3 5
11	9	2+ to 3+ 5 col 7 col	8	1	1	3.5
12	3	2 col 2 col 12 col.	2	1	1	15
13	3	2+ to 4+	3	0		
Totals	*109*		*85* (78%)	*24* (22%)		

taining 25 or more μg/ml. She estimated that these relatively resistant variants "occur infrequently, possibly once in several thousand times."

Youmans and Williston (28) found isolated streptomycin-resistant variants in cultures from nine of fourteen patients. Of the thirteen colonies obtained, four were resistant to 100 or more μg/ml and four were resistant to 25 or 50 μg/ml. Their studies were carried out with recently isolated, pure cultures of tubercle bacilli.

Yegian and Vanderlinde (27), working with pure cultures of H37Rv, discovered that about 6 out of 100 billion organisms were resistant to 1 μg/ml, and only about 1 5 out of 300 billion were resistant to 100 μg/ml.

In a study from Trudeau Sanatorium (4), specimens of concentrated and digested sputum from sixty-three patients were each planted on a series of tubes of solid medium containing 3 5, 15, 30, 200, and 500 μg/ml of streptomycin, and on two control tubes without streptomycin. All the cultures were highly positive. Fifty-four (86 per cent) showed no growth in any of the tubes containing streptomycin, and nine (14 per cent) produced one or more colonies in the streptomycin tubes. Of the nine cultures that demonstrated these variants, eight produced only one to three colonies in the tube containing 3.5 μg/ml of streptomycin, and one culture produced three colonies in 3 5 μg/ml, two colonies in 30 μg/ml, and one colony in 500 μg/ml. From these results, it appeared that some patients might produce cultures containing more resistant variants than others. To extend further these observations, repeated cultures at frequent intervals were obtained from thirteen patients, most of whom had highly positive specimens regularly (4). The results are shown in table 19

It will be seen from the above table that 78 per cent of the cultures showed no growth in any of the tubes except the controls, whereas 22 per cent produced colonies in the streptomycin tubes. Most of the cultures that contained resistant variants came from two patients, numbers 6 and 7. Almost all the variants appeared in the tube containing 3.5 μg/ml. Only two colonies out of 109 cultures were able to grow on the medium containing 15 μg/ml

Sensitivity tests in Tween-albumin medium were performed on forty-seven of the isolated variants that had appeared in the 3 5 μg/ml tube (4). Two proved to be sensitive to 1 0 μg/ml, ten to 2.5 μg/ml, twenty-three to 5.0, nine to 10, and three to 250 or more μg/ml. Of the two colonies from the 15 μg/ml tube, one was sensitive to 250 μg/ml in liquid medium, and the other was resistant to 1,000

These results indicate that the number of relatively resistant variants of tubercle bacilli, as determined by direct inoculation of solid medium with the processed sputum, is very small, and that certain patients show these variants with greater regularity than others It remains to be seen whether

pendent tubercle bacilli. A number of factors involved in this problem, which have an important bearing on the usefulness and the limitations of streptomycin as a therapeutic agent in tuberculosis, are discussed.

If the obstacle of streptomycin resistance could be overcome, the efficacy of the drug would be markedly increased. Investigations are now in progress to determine whether intermittent administration, or combination of streptomycin with such tuberculostatic agents as the sulfones or para-aminosalicylic acid, will delay the onset of streptomycin resistance. The shortening of a course of therapy for pulmonary tuberculosis to 4 or 6 weeks is already becoming standard practice to diminish the number of resistant strains, although it is realized that this limited period of treatment may not produce maximum results.

Further investigations should be made of the sensitivity of tubercle bacilli recovered from tissues removed at autopsy from various sites and types of lesions, along with careful microscopic examination of the tissues from which the organisms are isolated. The results of such observations may reveal whether resistant organisms emerge more readily from one type of lesion, or from one type of tissue, than from another.

The great variation in the development of drug-resistant tubercle bacilli exhibited by a large group of patients demands further study. Preliminary impressions derived from researches in the factors involved in this variation indicate that the location and the type and the extent of disease play an important role. For example, patients who have definite pulmonary cavitation are more likely to produce resistant cultures than are those without cavity (4, 32), and those who start treatment with high bacillary count in the sputum (Gaffky IV to X), show an increased tendency to yield drug-resistant cultures during streptomycin therapy over those patients with but few organisms in the sputum (Gaffky 0 to III) (4). Also, tubercle bacilli lodged in the central nervous system become resistant less readily than do those in the lungs.

What is the mechanism involved in the emergence of drug-resistant cultures *in vivo?* Is it merely a selective propagation of streptomycin-resistant variants that are already present in small numbers in any population of organisms—the sensitive individuals being eliminated by virtue of the suppressive action of the antibiotic? The data presented in this chapter would indicate that the problem is more complicated than this supposition would suggest

Futher study of the tendency of streptomycin-resistant tubercle bacilli to emerge readily in humans and mice, as contrasted with the infrequency of their appearance in guinea pigs, may lead to a better understanding of drug resistance.

these particular patients, when given streptomycin, will yield drug-resistant cultures more rapidly and with greater uniformity than others. It was demonstrated that cultures from the two patients, numbers 6 and 7, previously referred to, became resistant *in vitro* at the same rate as those from other patients in the study (4).

Williston and Youmans (10) reported on the correlation between the development of resistance in seven patients treated with streptomycin, and the ease with which the tubercle bacilli isolated from each patient before treatment could be made resistant *in vitro*. All seven eventually yielded streptomycin-resistant strains, but the organisms from only three of them acquired the ability to grow in more than 1,000 μg/ml after prolonged exposure to the drug *in vitro*; the pretreatment cultures from three of the patients acquired only fourfold increase in resistance, and the bacilli from one patient were sensitive to 50 μg/ml after *in vitro* exposure for 100 days.

Streptomycin-dependent Variants

It has been observed that many different types of bacteria produce variants that are dependent on streptomycin for their growth. Yegian and Budd (29) have reported such dependent variants in a culture of *M. ranae*, an acid-fast microorganism originally isolated from the liver of a frog.

Spendlove and others (30) reported that they had isolated a strain of *M. tuberculosis* var *hominis* the growth of which was markedly enhanced by the presence of streptomycin. This strain came from a patient with pulmonary tuberculosis on the 96th day of therapy with the drug. Another culture from this patient, obtained 4 months later, again revealed better growth on media containing streptomycin than on media without the drug. Guinea pigs infected with this streptomycin-enhanced culture and treated with the drug, succumbed sooner than the untreated controls (35).

Lenert and Hobby (31) found what they considered to be streptomycin-dependent organisms from thirteen of 196 mice infected with the H37Rv strain and treated with streptomycin for 31 days.

Although these reports indicate that dependent tubercle bacilli may be found, their occurrence is evidently very rare. At the Trudeau Laboratory (4), several thousand cultures isolated from patients during and after streptomycin therapy have been tested for drug sensitivity, yet no clear-cut instance of dependency has been observed.

Discussion

This chapter presents an evaluation of the data so far available concerning the emergence and the significance of streptomycin-resistant and -de-

pendent tubercle bacilli. A number of factors involved in this problem, which have an important bearing on the usefulness and the limitations of streptomycin as a therapeutic agent in tuberculosis, are discussed.

If the obstacle of streptomycin resistance could be overcome, the efficacy of the drug would be markedly increased. Investigations are now in progress to determine whether intermittent administration, or combination of streptomycin with such tuberculostatic agents as the sulfones or para-aminosalicylic acid, will delay the onset of streptomycin resistance. The shortening of a course of therapy for pulmonary tuberculosis to 4 or 6 weeks is already becoming standard practice to diminish the number of resistant strains, although it is realized that this limited period of treatment may not produce maximum results.

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CHAPTER 12

THE MODE OF ACTION OF STREPTOMYCIN

Streptomycin[1] is a complex organic substance which in low concentrations exhibits a remarkable antimicrobial action against many bacteria. The mechanisms by which this agent acts on the bacterial cell are not known. Aside from a purely didactic interest which may exist in these mechanisms, there is a possibility that their elucidation may be useful in development of better chemotherapeutic agents and possibly may indicate more efficient uses of streptomycin itself.

Our present knowledge concerning the mode of action of streptomycin is fragmentary. Relatively few investigations have been planned specifically to gather information on this subject. Nevertheless, many observations, made incidental to other problems under study, seem pertinent to the overall picture. It must be recognized in evaluating these studies that the extreme complexity of the living cell and the interdependence of its functions in certain instances make it difficult, and indeed hazardous, to draw conclusions concerning apparent cause-effect relationships.

FACTORS INFLUENCING STREPTOMYCIN ACTIVITY

The activity of streptomycin is influenced by its concentration and by environmental variables. To evaluate critically data bearing on the mode of action of streptomycin, it is essential that the effects of these variables be understood No evidence has been forthcoming to indicate that the mode of action of streptomycin against susceptible bacteria is different *in vivo* from that *in vitro*, and usually it is assumed tacitly—an assumption which conceivably may be in error—that chemotherapeutic drugs have the same mode of action under both conditions

[1] It has been suggested (1) that the term *streptomycin* be reserved for N-methyl-L glucosaminido-streptosido-streptidine, the predominant form of several closely related substances found in streptomycin concentrates The great majority of work reviewed in this chapter was carried out with preparations, the chief antibiotic principal of which was undoubtedly "*streptomycin*," but which also contained varying amounts of other forms of streptomycin and undefined impurities Unless otherwise specified, *streptomycin* as used in this review refers to these partly purified concentrates

Concentration of streptomycin

All other environmental variables being constant, the activity of streptomycin varies directly with concentration. It has been observed that the rate of increase in number of bacteria begins to decrease at a certain concentration of streptomycin and continues to decrease as the concentration of streptomycin is increased. This may be explained, on the one hand, on the basis that the mean generation time (the average time for one cell division) is affected by streptomycin, the time increasing with increasing concentrations of the antibiotic. On the other hand, since the susceptibility of individual cells to streptomycin varies over a wide range, it is conceivable that at the concentration of the antibiotic in which the inhibitory effect begins to appear, division of the most sensitive cells is completely blocked, and as the concentration of streptomycin is increased, more and more cells are inhibited. The effect of the concentration of drug would be apparent rather than real if the latter explanation were correct for streptomycin. Such a nearly-all-or-none effect is exerted by certain cell inhibitors, apparently by affecting primarily the lag phase of growth. Because of the difficulty in observing division of individual bacterial cells, one cannot easily determine which, if either, of these explanations may be correct. Data compatible with the hypothesis that streptomycin may prolong the lag phase of the bacterial growth cycle have been presented (2). This is a crucial point bearing on the mode of action of streptomycin and deserves further study (fig. 32).

Size of inoculum

Aside from concentration of the drug, one of the most important factors influencing the activity of streptomycin *in vitro*, and presumably *in vivo*, is size of inoculum (fig. 33) The larger the size of the inoculum, the greater the concentration of streptomycin required to inhibit multiplication completely (2, 3) This is due apparently to the fact that in any bacterial population there is a wide range of sensitivities to streptomycin; indeed, this range is probably greater for streptomycin than for any other cellular inhibitor studied to date Bacteria do not produce an antagonist for streptomycin, streptomycin is not destroyed in their presence, nor is a significant amount of streptomycin removed from the medium during growth (2, 4).

Environmental constituents

Streptomycin is adsorbed by various substances, including unknown constituents of certain culture media (5), unidentified constituents of blood serum and plasma (5), cellulose, oxidized cellulose (6), and desoxyribonucleic acid and thymonucleoprotein (6, 7) Such adsorptions can affect the activity of the drug only insofar as they reduce the concentration of free

streptomycin in the medium. Since these reactions are only partial, other variables must be in proper relationship for the adsorptions to be critical for bacteriostasis. This fact may account in part for the conflicting reports on the antagonistic effect of serum (2, 4, 8).

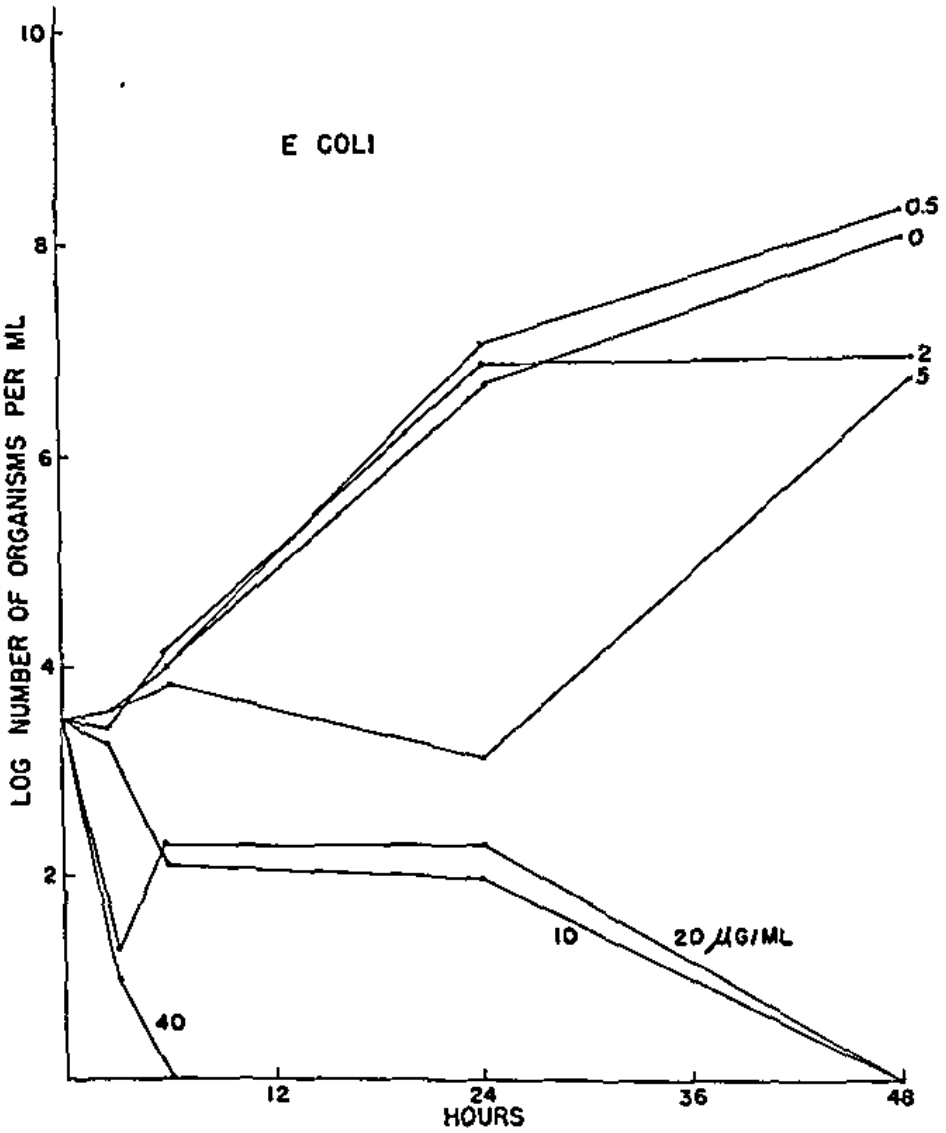

FIG 32 The effect of varying concentrations of streptomycin on its antibacterial action (8)

It is recognized that the value of streptomycin lies in its chemotherapeutic activity in vivo. The effect of serum and other body fluids is, therefore, of primary importance (table 20). Original reports on streptomycin suggested that substances may be present in the body which inhibit to some extent the influence of streptomycin on *S. typhosa*. *In vitro* studies, however, indicated that 10 per cent normal human serum enhanced the bacteriostatic action of streptomycin on this organism (4). In like manner, it has been observed by Berkman *et al.* (2) and others, working with strains

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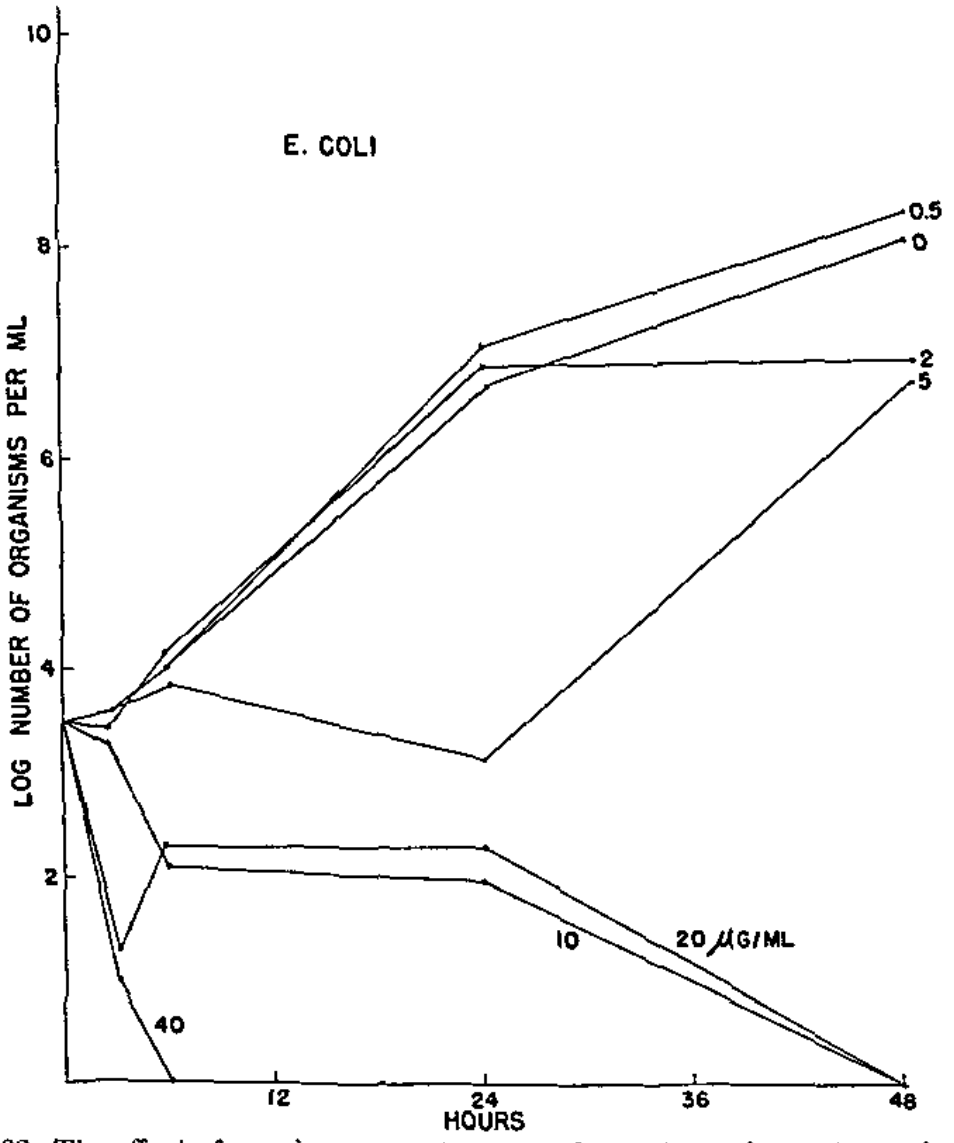

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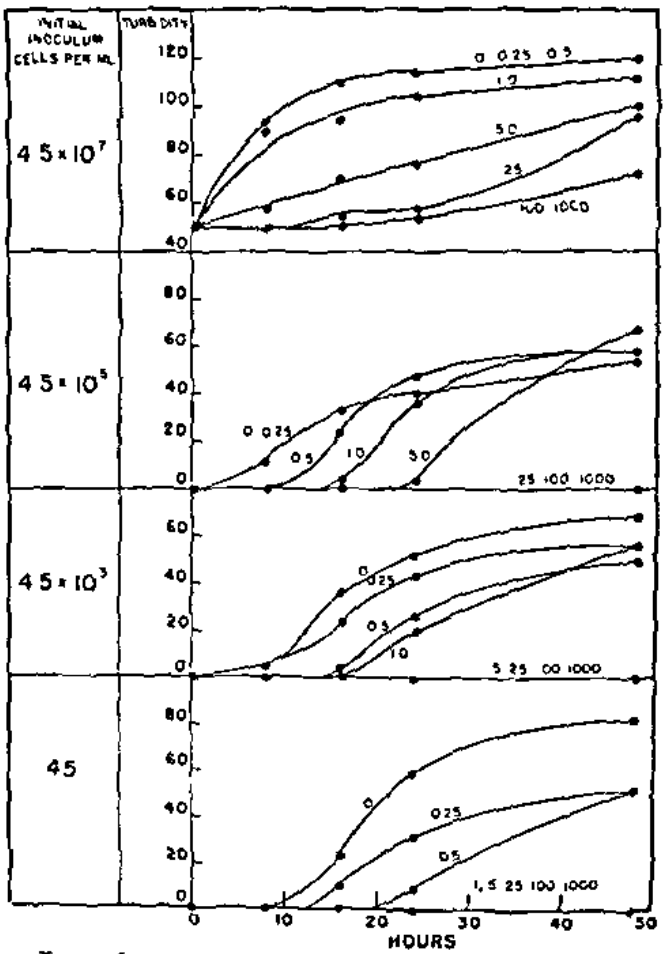

FIG 33 The effect of size of inoculum on the action of streptomycin (2).

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effect on the growth of the specific organism used, thus altering the number of organisms present and the ultimate effectiveness of the drug. The effect of serum on streptomycin activity would be dependent, then, upon the nature of the organism in question and its response to serum as a growth stimulant. If this is true, apparent disagreement between reports published on the effect of serum on the action of streptomycin can be explained in part by differences in the specific test organisms used in these studies. That this may not be the entire explanation is suggested by the fact that

TABLE 20

Effect of human serum on sensitivity of organisms to streptomycin (8)

STRAIN	SENSITIVITY IN μG/ML SERUM, PER CENT					
	0	1	5	10	20	50
E coli						
(W)	8 5	8 0	8 5	6 0	4.5	3.0
(Y)	5 0	5.0	4 0	4.0	4.0	2 0
(H)	7.0	7.0	6.0	5.0	4.0	2 0
(S)	>40.0	>40 0	>40.0	>40 0	32.0	8.0
(Ga)	40 0	20.0	32.0	36.0	32.0	12.0
(U)	>40.0	32 0	>40 0	24.0	28.0	8 0
A. aerogenes	2.0	2 5	2.0	2 0	1.5	1.0
Kl. pneumoniae	1 0	1.0	1 0	<0 5	<0.5	<0.5
S. aureus						
(H)	16.0	>40.0	>40 0	40.0	40.0	20.0
(M)	5 0	7 0	10 0	5 0	3.0	3.0
(HS)	8 0	>10 0	10.0	>10 0	8.0	4 0
S hemolyticus						
(C203Mv)	8.0	>40.0	>40.0	>40.0	>40 0	>40 0
D pneumoniae						
(I/230)	4 0	36 0	40 0	32.0	32 0	16 0
(D/39)	4.0	20.0	24.0	20 0	20 0	8.0
(A66)	<1.0	40 0	20.0	24 0	20 0	8.0

Sensitivity = least amount of streptomycin causing complete inhibition of growth after 72 hours at 37°C.

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The activity of streptomycin is extremely sensitive to the concentration of salt in the environment. The addition of 2 per cent salt may increase the minimal inhibiting concentration of streptomycin 100-fold (2). Salt reversal has been observed with all organisms studied and with salts containing sodium, potassium, lithium, ammonium, barium, magnesium, and calcium as cations, and chloride, sulfate, tartrate, phosphate, acetate,

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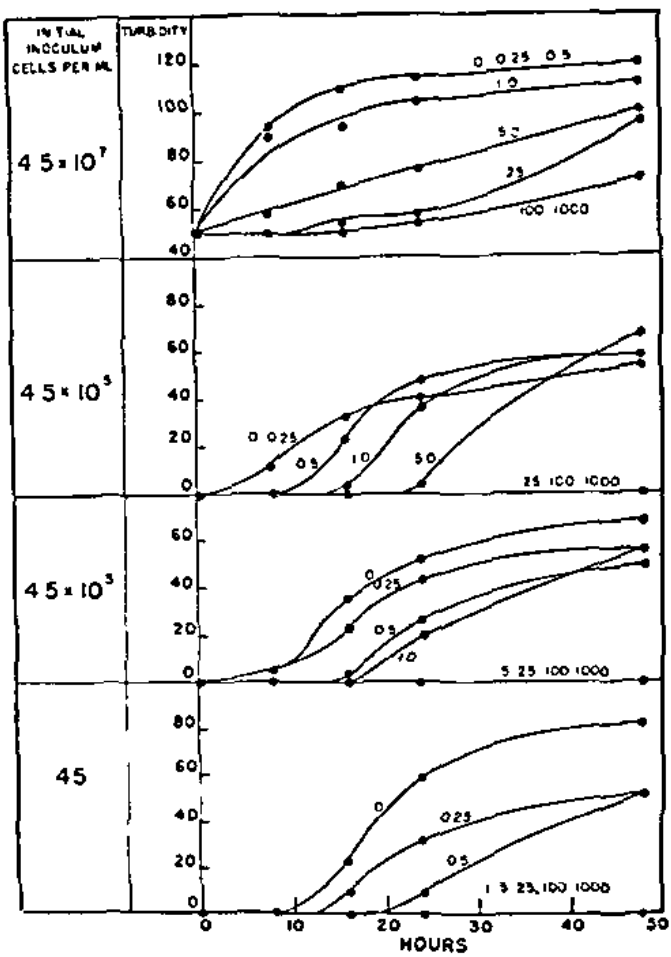

FIG 33 The effect of size of inoculum on the action of *streptomycin (2)*.

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(H)	7.0	7.0	6.0	5.0	4.0	2 0
(S)	>40 0	>40.0	>40.0	>40 0	32.0	8 0
(Ga)	40.0	20.0	32.0	36.0	32 0	12 0
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A. aerogenes	2.0	2.5	2 0	2 0	1.5	1 0
Kl. pneumoniae	1.0	1.0	1 0	<0 5	<0.5	<0.5
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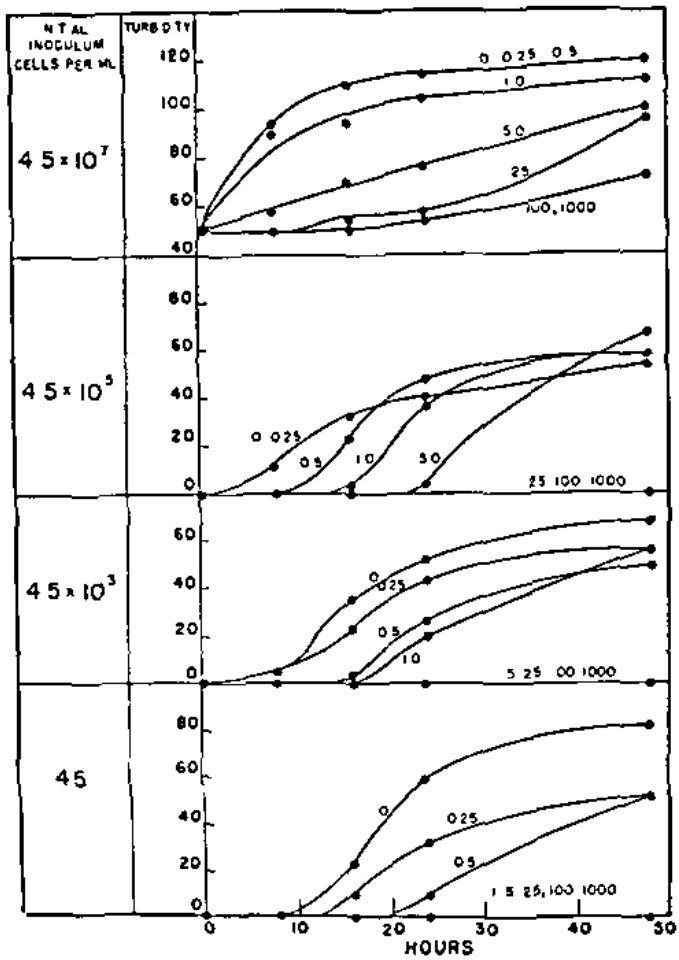

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(S)	>40.0	>40 0	>40.0	>40 0	32 0	8 0
(Ga)	40 0	20.0	32.0	36 0	32 0	12 0
(U)	>40 0	32 0	>40.0	24 0	28 0	8 0
A aerogenes	2 0	2.5	2.0	2 0	1 5	1.0
Kl pneumoniae	1.0	1.0	1 0	<0 5	<0.5	<0 5
S. aureus						
(H)	16.0	>40 0	>40 0	40.0	40.0	20.0
(M)	5.0	7 0	10.0	5 0	3.0	3 0
(HS)	8 0	>10 0	10.0	>10 0	8 0	4 0
S. hemolyticus						
(C203Mv)	8.0	>40.0	>40 0	>40 0	>40.0	>40.0
D pneumoniae						
(I/230)	4 0	36.0	40 0	32 0	32 0	16 0
(D/39)	4 0	20.0	24 0	20 0	20 0	8 0
(A66)	<1 0	40 0	20 0	24 0	20 0	8 0

Sensitivity = least amount of streptomycin causing complete inhibition of growth after 72 hours at 37°C.

serum apparently has a greater effect on the sensitivity of a single strain of *S. hemolyticus* (C_{203}Mv) to streptomycin than on the rate of growth of this organism (8).

The activity of streptomycin is extremely sensitive to the concentration of salt in the environment. The addition of 2 per cent salt may increase the minimal inhibiting concentration of streptomycin 100-fold (2). Salt reversal has been observed with all organisms studied and with salts containing sodium, potassium, lithium, ammonium, barium, magnesium, and calcium as cations, and chloride, sulfate, tartrate, phosphate, acetate,

of *S. aureus* and *E. coli*, that there is no decrease in the antibacterial action of streptomycin *in vitro* in the presence of serum. Hobby and Lenert (8), in a study of fifteen strains belonging to eight species of bacteria, observed a marked decrease (sixfold to fortyfold) in the sensitivity of *S. hemolyticus* and *D. pneumoniae* and a slight decrease (twofold) in the sensitivity of certain strains of *S aureus* to streptomycin when grown in the presence of 1 to 5 per cent human serum. No decrease in sensitivity to streptomycin

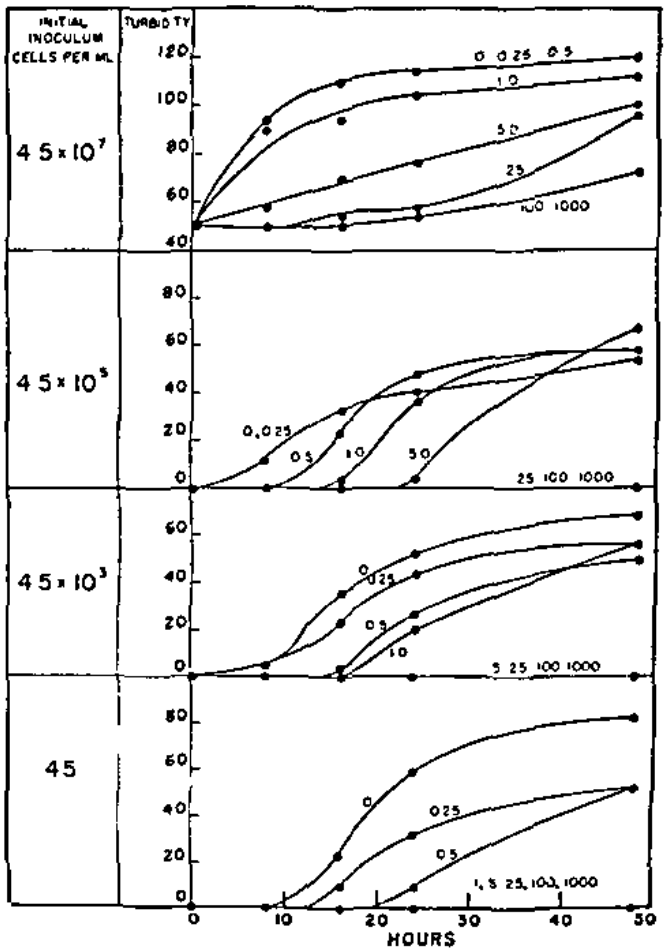

FIG 33 The effect of size of inoculum on the action of streptomycin (2)

was observed, however, with strains of *E coli*, *S typhosa*, *A. aerogenes*, or *Kl. pneumoniae* grown in broth containing this concentration of human serum In some instances, indeed, the bacteriostatic action of streptomycin was enhanced (for example, on *E. coli*) This is in agreement with the earlier observations of Reimann, Elias, and Price (9) on *S. typhosa*. It is well known that serum enhances the growth of hemolytic streptococci and pneumococci Furthermore, growth of *E. coli*, *A. aerogenes*, and *S. typhosa* is not enhanced by the presence of serum. It was suggested, therefore, that the effect of serum on the action of streptomycin may be due only to its

of streptomycin with the critical site of action. In this connection it seems significant that the adsorptions of streptomycin by bacteria (4), cellulose, desoxyribonucleic acid, and thymonucleoprotein (6, 7) can be inhibited completely or reversed by salts, whereas adsorption by serum components is not affected (9). It has been suggested that the antagonism to streptomycin manifested by organic salts may result from substitution in their presence of an alternative metabolic system which is resistant to streptomycin. This proposal would be more attractive if numerous inorganic salts were not equally potent antagonists. Sodium pyruvate, for example, on a weight basis exerts approximately the same antagonistic activity as sodium chloride. Futhermore, combinations of the two salts in a 1:1 ratio by weight have the same antagonistic activity as either salt alone, provided the concentrations of the individual salts are the same as those of the combinations. At present there is little evidence that the antagonisms manifested by the organic salts are related in any way to their role as metabolic substrates.

Glucose and certain other sugars have been reported by several groups of workers to antagonize the action of streptomycin (12). This antagonism has been attributed to an increased production of hydrogen ions, a decreased O-R potential, and an effect on the growth of the organism making higher concentrations of streptomycin necessary for inhibition of its growth. A recent report by Green and Waksman (11) has indicated that this antagonism is due neither to glucose *per se* nor to the acidity produced, but rather to the salt content of the medium and of the specific organic nitrogenous compounds in the medium.

The effect on streptomycin activity by substances influencing the reducing intensity of the medium and by certain other substances are discussed later.

pH effect

The activity of streptomycin is maximal at approximately pH 7.8 and decreases markedly below pH 7 0 (13) as shown in table 21. As determined from conductivity measurements streptomycin is highly dissociated at neutrality and above. Since this dissociation must decrease with decreasing pH, it seems likely that streptomycin is highly active in the dissociated state The possibility that the streptomycin base and the hydrogen ions compete on the surface of the bacterial cell for the active centers deserves consideration (11).

Locus of Action

As determined with the ultramicroscope, the calcium chloride double salt of streptomycin forms a colloidal sol in water rather than a true solution,

pyruvate, nitrate, lactate, citrate, fumarate, succinate, formate, malate, and maleate as anions (2, 7, 10, 11). There is evidence that if both anion and cation show reversal, their effects may be additive (7).

The effect of salt on streptomycin manifests itself in assay procedures (2, 11). Alterations in the diameters of zones of inhibition may be at least partly a function of the effect of salt on the diffusion rate of streptomycin through the assay medium. This consideration does not obtain in serial dilution assays or turbidimetric studies (fig. 34).

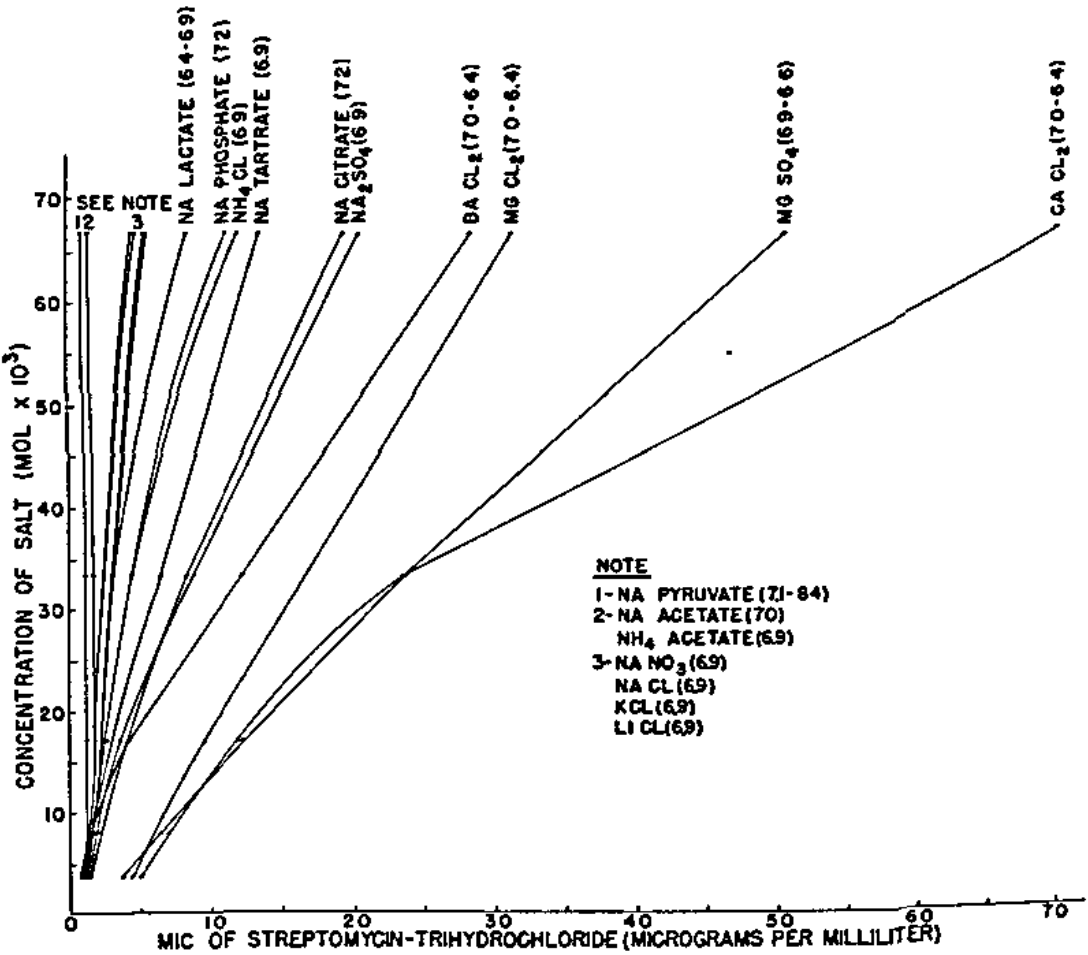

FIG. 34 Effect of salts and organic acids on potency of streptomycin (7)

No explanation exists for the antagonistic effect of salt on streptomycin. Electrophoretic studies have failed to reveal any evidence that the salt acts directly on streptomycin, producing an inactive complex, although this by no means proves that such a combination may not occur. The possibility that the antagonism may be a nonspecific stimulation of bacterial multiplication by the salt has been ruled out as a major factor by certain of the above reports. It is conceivable that the phenomenon may be the result of an interaction with the bacteria, in some way preventing the combination

of streptomycin with the critical site of action. In this connection it seems significant that the adsorptions of streptomycin by bacteria (4), cellulose, desoxyribonucleic acid, and thymonucleoprotein (6, 7) can be inhibited completely or reversed by salts, whereas adsorption by serum components is not affected (9). It has been suggested that the antagonism to streptomycin manifested by organic salts may result from substitution in their presence of an alternative metabolic system which is resistant to streptomycin. This proposal would be more attractive if numerous inorganic salts were not equally potent antagonists. Sodium pyruvate, for example, on a weight basis exerts approximately the same antagonistic activity as sodium chloride. Futhermore, combinations of the two salts in a 1:1 ratio by weight have the same antagonistic activity as either salt alone, provided the concentrations of the individual salts are the same as those of the combinations. At present there is little evidence that the antagonisms manifested by the organic salts are related in any way to their role as metabolic substrates.

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As determined with the ultramicroscope, the calcium chloride double salt of streptomycin forms a colloidal sol in water rather than a true solution,

the particle size of the dispersed phase being approximately 65μ. Streptomycin passes readily through a cellophane dialysis membrane, however, indicating that the physical dispersion of streptomycin in water is such that particle size *per se* is not an obstacle to its passage through a cellular membrane.

Berkman, Henry, Housewright, and Henry (4) have studied the distribution of streptomycin between bacterial cells and the surrounding water phase. The results obtained led to the conclusion that streptomycin is adsorbed at the cell surface and that little or no streptomycin actually penetrates the cell membrane. This adsorption can be prevented or reversed by sodium chloride. Streptomycin-resistant cells behave identically to sensitive cells.

Studies in both humans and dogs have indicated that streptomycin is distributed as if it were present in extracellular water only.

TABLE 21

Concentrations of streptomycin necessary for partial inhibition of growth of S. aureus at different pH levels (12a)

Incubation 24 hours

pH	CONCENTRATION OF STREPTOMYCIN IN UNITS PER ML FOR	
	Complete inhibition	Partial inhibition
7.7	1.6	0.8
7.2	6.25	1.6
6.6	6.25	3.2
5.9	50.00	25.0
5.2	100.00	40.0

Inoculum 0.1 ml of 12-hour broth culture diluted 10 times.

Effect of Streptomycin on Bacteria

Like most other antibacterial agents, streptomycin manifests both bacteriostatic and bactericidal actions (3, 14). Predominance of one activity over the other in any particular instance appears to be dependent, in part, upon the time of contact with streptomycin (3), the organisms involved, the concentration of streptomycin, and the size of inoculum (8). Other variables such as temperature, pH, and age of culture are influential (8). Bactericidal action has been observed with bacteria in the resting state (8, 10, 15), the concentration of streptomycin required being higher than for multiplying cells (fig 35)

The observation that resting cells exposed to streptomycin do not develop resistance has led Hamre, Rake, and Donovick (15) to postulate that the bactericidal action on resting cells is different from that on multiplying cells.

This observation *per se* does not seem to be a convincing argument for this viewpoint. The fact that streptomycin-resistant cells are also resistant to

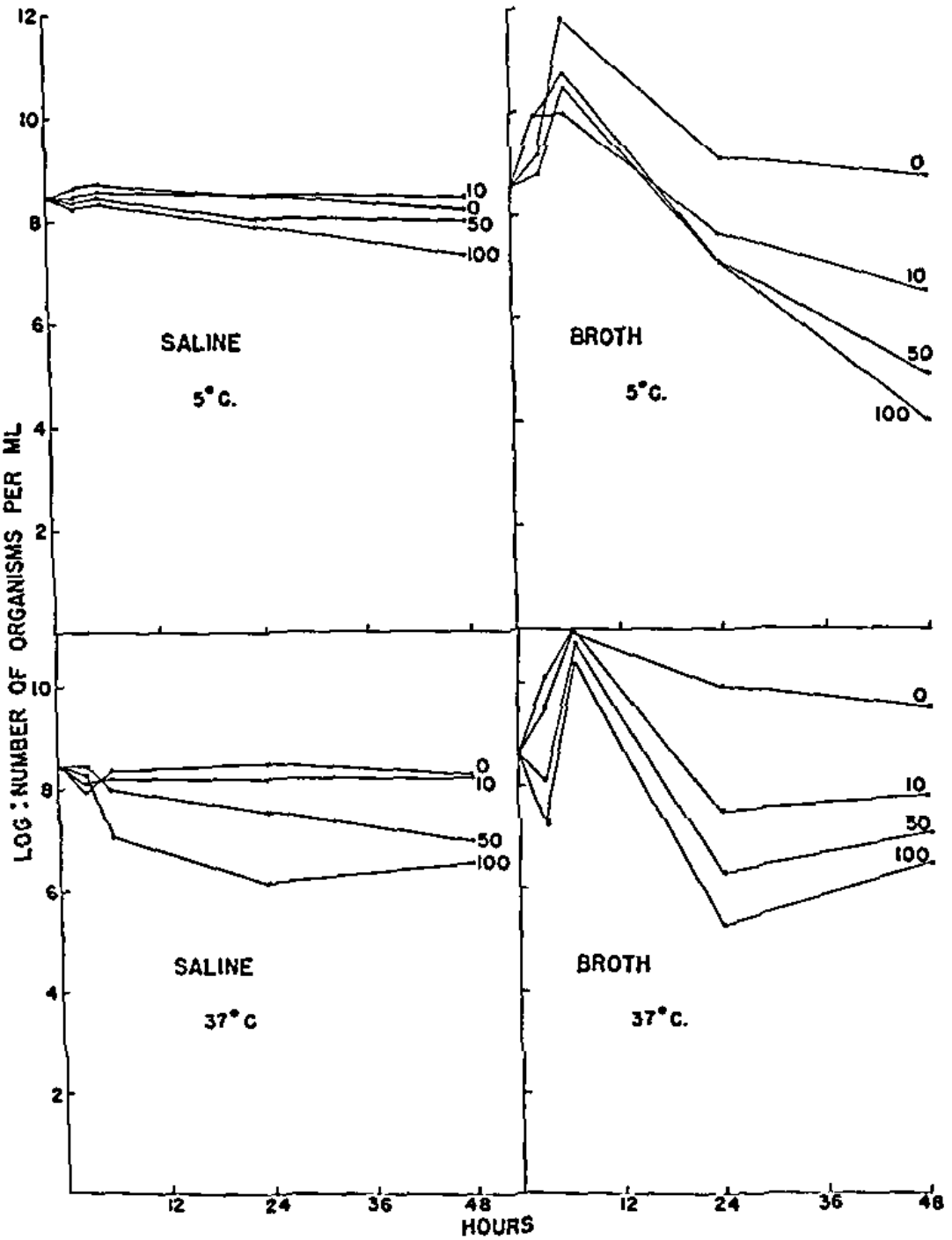

FIG. 35. Effect of streptomycin on high concentrations of organisms in stationary phase of *E. coli* (8).

the bactericidal action of streptomycin in the resting state has led Strauss to the opposite conclusion, that the bactericidal action on resting cells is the same as that on multiplying cells.

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The observation that resting cells exposed to streptomycin do not develop resistance has led Hamre, Rake, and Donovick (15) to postulate that the bactericidal action on resting cells is different from that on multiplying cells.

Thus there seems to be little doubt that development of resistance to streptomycin can occur exclusively by virtue of natural selective processes. It should be pointed out, however, that adaptation to streptomycin by cells originally sensitive has not been definitely ruled out as playing a minor role.

Several reports have appeared in the literature to the effect that, in some instances at least, the emergence of resistant cells in a given culture is accompanied by a decrease in the rate of growth of the culture, in its capacity to ferment, in its pigment production, and in its reducing intensity (21) Likewise, it has been demonstrated by Gezon and Cryst (22) that the development of resistance in cultures of hemolytic streptococci results in a change in colonial morphology and in a coincidental decrease in virulence and reduction in streptokinase (fibrinolysin) and streptolysin S production.

If the mode of action of streptomycin is actually associated with the metabolism of the bacterial cell, resistance to streptomycin should indicate a difference in metabolic activities, detectable perhaps by a comparison of the rates of utilization of various substrates by the susceptible and resistant strains. Henry, Henry, Berkman, and Housewright (23) have studied the rates of O_2 consumption and CO_2 production with numerous carbohydrate substrates in susceptible and resistant strains of *S. aureus*, *B. cereus*, and *S. dysenteriae sonnei*. Numerous quantitative differences in QO_2 values and anaerobic CO_2 production were observed between the susceptible and resistant strains of the three organisms, but no indication as to the cause for resistance or the mode of action of streptomycin could be derived from these differences, since none was consistent among the three organisms These changes did not occur consistently in the same direction, furthermore, and there were as many increased values for the resistant strains as there were decreased values. This probably indicates that the interpretation of Seligmann and Wasserman (24) that resistant strains have "damaged" enzyme systems is unwarranted.

Exposure of bacteria in the resting state to streptomycin does not result in emergence of resistant cells (10). This fact does not preclude, however, the possibility of adaptations arising by development of alternative pathways of metabolism

Streptomycin-dependent Variants

Probably the most remarkable observation on streptomycin was that first reported by Miller and Bohnhoff (25), who demonstrated that bacteria could become dependent on streptomycin for growth. From each of eighteen strains of meningococcus they obtained two streptomycin-resistant variants. One, designated as *variant type A*, appeared in small and equal numbers on all concentrations, grew in large yellowish colonies on

Bactericidal action, by definition, means the irreversible inhibition of growth or killing of bacteria. Many cellular inhibitors will kill cells even in static concentrations if the time of contact is sufficiently long. An inhibited cell is not a normal cell and, unless it is able to readjust itself within certain limits of time (in which case it may become resistant), it may succumb. The conclusion that the bacteriostatic and bactericidal mechanisms of streptomycin are dissociated, therefore, is open to question.

Streptomycin exhibits a biphasic action *in vitro* (16), that is, it stimulates bacterial multiplication in subinhibitory concentrations. Failure to obtain this stimulation with *Kl pneumoniae* has been reported. Welch, Price, and Randall (16) were able to demonstrate this phenomenon *in vivo*. Streptomycin at a certain dosage increased the fatality rate of *S. typhosa* for mice, whereas in higher dosages protective effects were obtained This has definite implications in the clinical use of streptomycin. It is a phenomenon which is found commonly with cell inhibitors *in vitro* but is understood in very few cases.

Streptomycin, like many other antibacterial agents, produces an alteration in bacterial morphology coincident with bacteriostasis. These morphological changes vary with different bacterial species and are not observed with all bacteria. They are often observed, however, even in cultures of organisms resistant to the concentration of streptomycin used (16, 17, 18). Changes in morphology, when they occur, may be accompanied by changes in staining characteristics (18).

Differences between Streptomycin-susceptible and Streptomycin-resistant Variants

The subject of the development of resistance to streptomycin is fully covered in another chapter of this book, but certain observations appear to be germane here Resistant strains can be produced by exposing parent susceptible strains to inhibitory concentrations of streptomycin. There has been one report that resistant strains can be produced also by exposure to subinhibitory concentrations. This suggests that the organism may be affected before inhibition of growth becomes apparent and that elimination of susceptible cells may not be the only cause for the apparent development of resistance From the results of a careful study of ten strains of type b *H influenzae* reported by Alexander and Leidy (19) it is apparent that emergence of resistance could be solely the result of a selective process. In large samples of initial bacterial populations, these investigators found resistant variants in all strains studied. The incidence of resistant cells varied from 1 in 1.1 billion to 1 in 13.8 billion organisms. This has been confirmed by Iverson and Waksman (20), who found one resistant cell present among 1.5 billion normal sensitive cells in broth cultures of *E. coli*.

In a comprehensive study on the distribution of dependent cells of *E. coli* in a broth culture of this organism, Iverson and Waksman (20) found that one dependent cell was present among each 1.5 billion normal sensitive cells. That streptomycin, and not an impurity, is required for growth of streptomycin-dependent organisms is suggested by the fact that growth of the strain is supported by numerous commercial preparations of streptomycin (25) by pure N-methyl-L-glucosaminido-streptosido-streptidine, pure mannosidostreptomycin, and pure dihydrostreptomycin (27) but not by streptomycin inactivated by cysteine or hydroxylamine (25) or by streptidine or streptamine (27). Furthermore, streptothricin is incapable of supporting growth of at least certain streptomycin-dependent strains (20). There appears to be a striking quantitative correlation between the stimulatory action of streptomycin for the streptomycin-requiring strain and its antibacterial action against the parent strain. Paine and Finland (28) have reported that the maximal concentration of streptomycin permitting growth of the sensitive parent strain and the minimal concentration supporting growth of the dependent variant are approximately equal in each case.

The acquisition of dependency on a cellular inhibitor is not without precedent, for example, *N. crassa* and sulfanilamide. If it is assumed that streptomycin inhibits the metabolism of susceptible bacteria, resistance to the drug may possibly indicate the presence in the resistant cell of an alternative metabolic pathway that is insensitive to streptomycin. The sensitive metabolic pathway present in susceptible cells may be present also, but in the ordinary resistant cell not dependent on streptomycin it is of no great consequence whether this pathway is suppressed or not. It is theoretically possible that in certain cases (streptomyin-dependent cells), however, release of the suppression of this pathway by removal of streptomycin may so upset the metabolic balance of the cell as to result in its death. There are no data at present, however, to support such a hypothesis as this.

Proposed Mechanisms of Action of Streptomycin

Streptomycin and sulfhydryl groups

It has been reported many times that the antimicrobial activity of streptomycin decreases as the Eh of the system decreases Such a decrease can arise either from the endogenous reducing intensity developed within the organism itself (24) or through externally added reductants such as cysteine and other sulfhydryl compounds, stannous chloride, sodium bisulfite, and sodium thiosulfate (29). Anaerobiosis also decreases the activity of streptomycin, although Geiger, Green, and Waksman (30) have attributed this to a pH effect.

streptomycin-free and streptomycin-containing media, and retained the original virulence for mice possessed by its parent strain. The other, *variant type B*, appeared in greatest numbers on concentrations between 100 and 400 μg of streptomycin per milliliter. Its colonies varied in size and color depending upon the concentration of streptomycin in which they were developed and were dependent on streptomycin for multiplication. This dependency was demonstrable not only *in vitro* but also *in vivo*, since the organism exhibited no virulence for mice unless streptomycin was administered to the animals following infection. Both variants retained the characteristic sugar fermentations and type specificity of the parent strain.

The production of streptomycin-dependent strains of *E. coli*, *Ps. aeruginosa*, *B. subtilis*, *S. aureus*, *P. morgani*, *M. ranae*, *M. tuberculosis* var. *hominis* has been reported.

The differentiation between strains dependent upon streptomycin for growth and those the growth of which is merely enhanced by streptomycin is important in the evaluation of any studies utilizing these strains. An organism dependent upon streptomycin for growth is one which is incapable of multiplying, *in vitro* or *in vivo*, in the absence of N-methyl-L-glucosaminido-streptosido-streptidine or one of its derivatives (as mannosidostreptomycin or dihydrostreptomycin). Two strains of *M. tuberculosis*, the growth of which is enhanced by, but is not dependent upon, the presence of streptomycin have been isolated from human sputa by Spendlove, Cummings, Fackler, and Michael. (26). Furthermore, a strain of *M. tuberculosis* has been described by Lenert and Hobby which has remained dependent upon streptomycin even after repeated subculture *in vitro*, yet is highly virulent for experimental animals. It is apparent that this strain is dependent upon streptomycin for growth *in vitro*, yet is capable of multiplying *in vivo* in the absence of streptomycin.

Aside from the possible clinical significance of these findings, their relationship to the mode of inhibitory action of streptomycin is of interest. The suggestion that dependent variants utilize streptomycin as an essential metabolite or growth factor is, of course, an attractive one (28). Such a utilization, so far, has not been demonstrated conclusively. The isolation by Hobby *et al.* of a gram-negative microorganism highly resistant to streptomycin and also capable of growing in solutions of highly purified streptomycin in distilled water, however, has been reported. The distilled water used in this study had a total solid content of less than 10 ppm and supported growth to a slight extent only. The addition of streptomycin to this distilled water produced a marked increase in the rate of multiplication of the organism, suggesting the possibility that streptomycin actually may be utilized during growth.

cleic acid in the cell. These observations led Donovick and co-workers to the conclusion that streptomycin does not act by combining with desoxyribonucleic acid in the cell. Furthermore, as previously mentioned, streptomycin combines with other substances, such as certain proteins and cellulose.

Krampitz, Green, and Werkman (33) reported that streptomycin inhibits the oxidation of ribonucleic acid by a strain of *S. aureus*, whereas Henry *et al.* (23), working with a different strain of *S. aureus*, were unable to obtain evidence of such inhibition.

Streptomycin and essential metabolites

If streptomycin inhibits the formation or the utilization of a substance essential for bacterial multiplication, then addition of this substance should antagonize streptomycin, noncompetitively in the former case and competitively in the latter. The antagonism by salts can scarcely be specific, since many are active in this respect.

Fitzgerald, Bernheim, and Fitzgerald (34) working with one strain of *E. coli* and two strains of *M. tuberculosis*, have reported antagonism of streptomycin by urea, xanthine, uric acid, allantoin, alloxan, and parabanic acid. Reversal by urea has also been reported. Tytell and Tytell (35) had previously reported that streptomycin inhibited the glucose dehydrogenase activity of *C. perfringens* and that this inhibition could be reversed by adenine, guanine, hypoxanthine, xanthine, and methionine. Recently, however, Fitzgerald *et al.* (34) reported that reversal by urea is due not to the urea *per se* but to cyanate formed in the process of sterilization by autoclaving. He believes that the cyanate probably inactivates streptomycin directly, possibly by combining with free amino groups.

Wallace, Rhymer, Gibson, and Shattuck (36) concluded from their experiments that there is something present in brain-heart-infusion broth, and not in nutrient broth, which interferes with the action of streptomycin. The active substance proved to be extractable with methyl alcohol, rapidly dialyzable, destroyed by trypsin hydrolysis, not adsorbed by charcoal, and present in large amounts in brain tissue (37). Solubility of the active substance in both ether and water and occurrence of the substance in brain and phytone suggested to Rhymer, Wallace, Byers, and Carter (37) that the substance may be lipositol. Test of one sample showed lipositol to be a potent antagonist, and the presence in lipositol of an inositol-galactose structure has suggested the possibility of a metabolite-antimetabolite relationship with streptomycin. Rake (27) reported that some lipositol samples showed slight activity in supporting growth of a streptomycin-requiring strain of *E. coli*. Further work is needed to de-

Cavallito and his coworkers have studied the antagonistic effects of various thiols on the activities of several antibiotic substances and have concluded indirectly that the point of attack by the antibiotics is on cellular thiol groups. Cavallito (31) has stated that streptomycin reacts readily with sulfhydryl compounds containing basic amino groups in the vicinity of the sulfhydryl group and that this reaction may occur in two ways: first, with essential sulfhydryl groups of enzymes; second, with sulfhydryl in cysteinyl residues as these are joined at the end of a growing polypeptide chain during protein anabolism, thus blocking growth of protein by producing cysteine "dead ends." He believed further that chemical reaction with sulfhydryl groups may be preceded by adsorption in their vicinity. That the neighboring amino groups may also be involved has been suggested (32).

The importance of free sulfhydryl groups to the activity of certain purified enzyme systems and to the normal functioning of the cell as a whole is well established. As yet, however, direct evidence that streptomycin effects its bacteriostatic activity through reaction with cellular sulfhydryl groups or, in fact, does react with them at all, is still lacking. Henry, Henry, Berkman, and Housewright (23) obtained no inhibition of activity of urease, succinoxidase, or carboxylase, enzymes requiring free sulfhydryl groups for full activity, even by 500 μg of streptomycin per milliliter These observations were made with purified enzyme systems, and it is recognized that inhibitor studies with such systems do not necessarily indicate that a similar result will be had when the enzyme is situated in its normal protoplasmic environment These negative results do indicate, however, that streptomycin is not a powerful sulfhydryl reactant.

Streptomycin and nucleic acids

Cohen (6) has observed that streptomycin combines with desoxyribonucleic acid, but not with ribonucleic acid, to produce polymeric compounds that actually precipitate, a reaction that can be reversed by the addition of sodium chloride These observations were confirmed by Berkman *et al.* (4), who also found that a similar complex was formed with calf thymus nucleoprotein Cohen suggested the possibility that the *in vitro* reactivity of streptomycin with desoxyribonucleic acid is related to its bacteriostatic action According to Donovick, Bayan, Canales, and Pansy (7), the order of activity of salts in reversing the desoxyribonucleic-acid-streptomycin complex differs from that of reversal of multiplication inhibition They observed depolymerization by desoxyribonuclease of desoxyribonucleic acid precipitated by streptomycin, resulting in disappearance of the precipitate, yet the minimal inhibiting concentration of streptomycin for *Kl. pneumoniae* was the same with or without added desoxyribonu-

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termine whether lipositol itself is the active principle and the exact nature of the antagonism reported.

Streptomycin and oxidation-reduction reactions

Relatively little information has accumulated so far on the effects of streptomycin on bacterial metabolism. Fitzgerald *et al.* (34) have reported that streptomycin in a concentration of 10 μg/ml completely inhibited the oxidation of benzoic acid by a susceptible strain of mycobacteria, whereas 100 μg/ml had no such effect on a resistant strain. With another susceptible strain, however, no inhibition was obtained. Further study revealed that streptamine and streptidine had no effect on the oxidation of benzoic acid, and there was always a latent period before inhibition of oxidation of benzoic acid appeared, that results obtained by varying the substrate concentration indicated competitive inhibition, and that the oxidations of trehalose, mannitol, fatty acids, and mucic acid were affected less than that of benzoic acid or not at all (the oxidations of phenol and tyrosine were inhibited in one species). Since there was a parallelism between the inhibitions of oxidation and growth, it was believed that this may be an important mechanism in the bacteriostatic action of streptomycin. It was recognized, however, that it undoubtedly is not the only one, since other organisms do not oxidize benzoic acid. More recently obtained evidence has indicated (34) that benzoic acid oxidase *per se* is relatively insensitive to streptomycin and that the inhibitions observed are due to suppression of the formation of the enzyme.

Failures to obtain streptomycin inhibition of carbohydrate metabolism in resting bacteria have been reported by several investigators No inhibition of the oxidation of pyruvate, fructose, and acetate in mycobacteria was observed with streptomycin in a concentration of 100 μg/ml (34). Working with *E. coli*, Geiger (38) found no effect of streptomycin on oxidation of glucose, lactate, glycerol, succinate, malate, fumarate, oxaloacetate, and pyruvate, and no effect on SO_2 production or H_2 production under anaerobic conditions Hirsch and Dosdogru (39) observed no inhibition of O_2 consumption by resting cells of a strain of staphylococci with lactate as substrate. Benham (40) reported that addition of 500 to 1,000 μg of streptomycin per milliliter to *S. typhosa* resulted in an immediate and marked increase in the rate of O_2 consumption, which abated after 2 hours and at 6 hours was less than the controls. With glucose added as substrate the percentage of theoretical O_2 consumption in the presence of streptomycin rose to 100 Benham concluded that utilization of carbohydrate appears to be more complete and more rapid when streptomycin is present (table 22).

Henry *et al.* (23) have made a study of the effect of streptomycin on certain purified enzyme systems and on the carbohydrate metabolism of resting cells of susceptible and resistant strains of *S. aureus*, *B. cereus*, and *S. sonnei*. Streptomycin in very high concentrations did not inhibit catalase, carbonic anhydrase, cytochrome, cytochrome-oxidase, succinoxidase, carboxylase, urease, or trypsin. In the bacterial experiments, streptomycin in concentrations just bacteriostatic inhibited noncompetitively the aerobic oxidation of glycerol and lactate by *S aureus* (table 22); of glycerol, lactate, glucose, pyruvate, ethanol, and acetate by *B. cereus*; and of glycerol, lactate, pyruvate, succinate, and acetate by *S. sonnei*. Anaer-

TABLE 22

Effect of streptomycin on the aerobic oxidation of glycerol and lactate by S. aureus (23)

SUBSTRATE (0.5%)	STRAINS	SM	AVERAGE PERCENTAGE INHIBITIONS DURING TIME PERIODS (HR) INDICATED			
			0–0.5	0.5–1	1–2	2–3
		μg/ml				
Glycerol	S*	1	0	10	14	20
		10	0	15	26	32
		100	0	23	35	42
	R	100	0	0	0	0
		1,000	0	0	0	20
Lactate	S	10	0	7	15	20
		100	0	14	22	25
	R	1,000	0	0	0	0

* S = susceptible strain; R = resistant strain.

obically, streptomycin inhibited the formation of CO_2 from glucose and pyruvate by *B. cereus* and from pyruvate by *S. sonnei*. Resistant strains of these organisms were affected only slightly or not at all by high concentrations of streptomycin. The percentage of theoretical O_2 consumption did not increase in any instance, nor did the R.Q. values change. Direct chemical analysis of supernatant fluids from experiments with *B. cereus* and *S. aureus* showed that every instance of streptomycin inhibition resulted in an increased accumulation of acetic acid. In almost every instance where inhibition appeared, it did so only after a lapse of time. The question of cell permeability having been ruled out experimentally, it was believed that this lag period for development of inhibition may represent the time during which some substance essential for metabolism is being deple-

ted and not being replaced. Streptomycin would then be blocking the formation of this essential substance, perhaps an enzyme. Although it was admitted that one could not conclude from these experiments that the inhibitions observed bear a causal relationship to the bacteriostatic action of streptomycin, it was pointed out that the following facts were compatible with such a hypothesis: first, the inhibitions in the susceptible strains were brought about by concentrations of streptomycin which just caused bacteriostasis; second, the same functions in resistant strains were affected appreciably less or not at all by much higher concentrations of streptomycin; third, the pH activity curves for streptomycin were very similar in the two cases, fourth, salts reversed both phenomena; and fifth, streptomycin samples of varying purity produced the same degree of inhibitions, indicating that these inhibitions probably were not due to one or more impurities present.

The above work has been extended to multiplying cells of *S. sonnei* with pyruvate and glucose as substrates, the addition of either of which to the basal medium used initiated multiplication (41). Inhibition of rates of multiplication and of O_2 consumption (per cell) usually increased with time following a lag of approximately 1 hour before inhibition developed. The inhibition of O_2 consumption was quantitatively less than the inhibition of multiplication The over-all inhibition of O_2 consumption closely paralleled the inhibition of substrate utilization. Quantitation of the acetic acid formed during the experiments tended to indicate an increase with increasing inhibition by streptomycin, the amounts formed, however, were so small as to lead to considerable experimental error in their determination. The percentage of theoretical O_2 consumption did not change significantly during streptomycin inhibition.

The effects of streptomycin on the total nitrogen, phosphorus, and reducing substance content of multiplying cells of *B. cereus* were also studied Where inhibition of multiplication occurred with streptomycin the nitrogen and phosphorus content was lower, and the reducing substance content was increased These changes are somewhat difficult to interpret with our present knowledge.

Geiger (38) observed that oxidation of amino acids by *E. coli* was facilitated by preincubation with fumarate or certain other carbon compounds, and that this increased ability of such cells to oxidize amino acids was inhibited after a lag period of 30 minutes to 1 hour by concentrations of streptomycin as low as 0.5 μg/ml It was suggested that an unidentified intermediate may be formed during the preliminary oxidation of the carbon compounds, that this intermediate is involved in the subsequent amino acid oxidation, and that streptomycin inhibits the metabolism of amino acids in the presence of the intermediate Experimental data indicated

that malate, succinate, oxaloacetate, glucose, lactate, and glycerol, but not pyruvate, are capable of replacing fumarate and possibly of producing the hypothetical intermediate mentioned above. Experiments indicated that the intermediate was located in the cells, that it was not CO_2 or oxaloacetate, and that it may be a phosphorylated intermediate, since phosphate was necessary for its formation. Since CO_2 production and O_2 consumption during oxidation of fumarate were not affected by streptomycin, it was believed more likely that streptomycin blocked utilization of the intermediate or promoted its decomposition, rather than prevented its formation. Geiger was unable to show any inhibition of transaminations with 80 μg of streptomycin per milliliter. From these findings it was considered probable that the effect of streptomycin upon *E. coli* involves, at least as one mechanism, interference with amino acid metabolism.

Related Forms of Streptomycin

It has been established both by chromatography and by the Craig technique of counter-current distribution that streptomycin concentrates contain two predominant substances, both active and related structurally. One, N-methyl-L-glucosaminido-streptosido-streptidine, appears to be the major component of crude and partly purified preparations. The other, mannosidostreptomycin, has been investigated and has been found to be a highly effective antibacterial agent. Whether these two streptomycins or their hydrogenated forms—dihydrostreptomycin and dihydromannosidostreptomycin—are alike in their modes of action is not known. Their antibacterial spectra are qualitatively the same.

It has been suggested that the activity of dihydrostreptomycin results from its oxidation to streptomycin by the bacteria. This has not been proved, however, and one must await further study before evaluating its mechanism of action.

Streptomycin residues, preparations remaining after removal of highly purified streptomycin from impure streptomycin concentrates and having antibacterial properties, have shown enhanced activity. The protective action of these residues against experimental tubercular infections in mice is greater than that of a comparable amount of either the N-methyl-L-glucosaminido-streptosido-streptidine or the mannosidostreptomycin present in them. Clarification of the effect of impurities on the action of streptomycin *in vivo* ultimately may alter our concept of the mechanism by which streptomycin acts within the host.

Discussion

The mechanisms by which antimicrobial agents function have been of interest to investigators in the field of biology for many years. Despite

numerous studies designed to elucidate the mode of action of these substances, there is still, in most cases, no clear conception of the actual steps involved. The complexity of living organisms and the technical difficulties in observing reactions within these cells have made it difficult, if not at times impossible, to determine the exact mechanisms involved.

It has been proved that certain factors affect the antimicrobial activity of streptomycin, and it has been suggested that certain other factors may influence its activity. It is recognized, however, that many of the data on the mode of action of this drug are still inconclusive.

Streptomycin is capable of both bacteriostatic and bactericidal actions. Bacteriostasis is the inhibition of cells normally capable of multiplication, and it seems quite possible that what is sought in studies on the mode of bacteriostatic action may be absent if resting cells are used. It has been demonstrated, for example, that sulfanilamide has no effect on the O_2 consumption of resting, unfertilized eggs of the sea urchin but inhibits the O_2 consumption of fertilized, multiplying eggs.

The acquisition of resistance to streptomycin or of dependency upon this drug probably is closely related to its mode of action. It is possible that the answer to one problem would clarify the other. A concomitant study of resistant strains derived from the susceptible strains used in research on the action of streptomycin would serve as a guide to whether the response to streptomycin which is measured is related to the bacteriostatic activity of streptomycin.

Most of the work reviewed herein was done before the heterogeneity of streptomycin concentrates was resolved and before purified compounds were available. Whether N-methyl-L-glucosaminido-streptosido-streptidine, mannosidostreptomycin, or the hydrogenated forms of these are alike in their modes of action is not known. Insofar as possible, therefore, future experimental work should be done with the purified forms.

It is evident from the reports discussed above that there are numerous cases of disagreement in results of different workers employing different organisms or even different strains of the same organism. It is usually assumed tacitly that the mode of action of an antibacterial agent is the same for different organisms; this conceivably may not be true in all cases. Whether this accounts for contradictions between reports or whether differences in the preparation of streptomycin or in the experimental conditions used are responsible cannot be determined. It would seem advisable, however, to examine as many organisms as feasible in any research of this nature.

The value of streptomycin lies in its *in vivo*, not in its *in vitro*, activity. It is assumed that the mechanism by which streptomycin acts on the bacterial cell itself is the same *in vitro* and *in vivo*. In evaluating the action

of this or other chemotherapeutic agents *in vivo*, however, the added effect of the defense mechanisms of the body must be considered.

The following available observations bear on the problem of the mode of action of streptomycin: streptomycin activity varies directly with concentration and inversely with hydrogen-ion concentration; little or no streptomycin enters the bacterial cell; the antibiotic is adsorbed by the bacterial cell, desoxyribonucleic acid, thymonucleoprotein, certain other proteins, and cellulose; it often exhibits a biphasic action; it is both bacteriostatic and bactericidal, depending on several factors; the bacteriostatic action of streptomycin appears after a lag period; its action is antagonized by most inorganic and organic salts and by many sulfhydryl compounds; bacterial strains develop resistance to streptomycin at a rapid rate and to a great degree; bacteria can become dependent on streptomycin for growth. It has been reported that streptomycin is antagonized by lipositol; it also has been reported, but not always confirmed, that streptomycin inhibits the metabolism of carbohydrates, ribonucleic acid, benzoic acid, and amino acids.

It is apparent that a definite conclusion as to the mode of action of streptomycin cannot be reached at present. It seems clear, however, that streptomycin inhibits certain bacterial enzymes, and it may be that the process of cell division or the synthesis of protoplasm is blocked by interference by streptomycin with one or more enzyme systems essential to these functions.

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CHAPTER 13

SYNERGISM BETWEEN ANTIBACTERIAL SUBSTANCES WITH SPECIAL REFERENCE TO STREPTOMYCIN

The term SYNERGISM when applied to antibacterial substances should connote the ability of two agents acting simultaneously to bring about bacteriostasis at individul threshold concentrations which are lower than could be accounted for by a mere summation of the individual effects of the discrete substances. Because practical difficulties may sometimes arise in establishing this criterion, the term, for the purposes of this discussion, is broadened to include those instances where the bacteriostatic effect *in vitro* or the curative effect *in vivo* of a particular drug is substantially increased by addition of another agent.

The occurrence of synergism has been recorded between pairs of various nonantibacterial drugs, between various insecticides, between fungicides, between various "germicides" with surface-tension depressants, and between phenols with the organic solvents acetyl methylcarbinol and diacetone alcohol. Current studies with sulfonamides, dyes, sera, and antibiotics will add to an already lengthy list of reported potentiation.

SYNERGISM BETWEEN MODERN CHEMOTHERAPEUTIC AGENTS

With respect to the modern chemotherapeutic agents, the possibility of synergism seems first to have been determined by Neter (1), who described an *in vitro* potentiation of sulfanilamide induced by azochloramid against group A hemolytic streptococci and enterococci. Neter indicated that his experiments were prompted by the work of Henderson and Gorer, which was confirmed by Mather, that a tenfold "synergic" curative response against "vibrion septique" in mice was effected by joint use of sulfapyridine and antitoxin or antibacterial immune sera. Schmelkes and Wyss (2) showed a similar "potentiated" rather than additive effect between azochloramid, wetting agents, and a number of sulfonamides against various species of bacteria from infected wounds. Skelton (3) confirmed the sulfonamide-azochloramid relationship *in vitro* against *S. agalactiae,*

but the combination proved harmful in attempts to control bovine mastitis.

Thatcher (4) reported a synergistic action between methylene blue or brilliant cresyl blue with sulfonamides against *E. coli* and *S. aureus*. Under specific conditions, sulfathiazole and methylene blue together were shown by Thatcher and MacLean (5) to be highly effective *in vivo* against *E. coli* in various urological habitats. Marked improvement by the combination over the effects of the individual agents used singly was frequently observed.

McIntosh and Selbie (6) found that acridine dyes and sulfathiazole were more effective in combination than when used alone for treatment of wounds infected with various anaerobic bacteria.

In vitro studies by Gershenfeld and Sagin (7) indicated a marked synergism between sulfonamides and both anionic and cationic detergents against *E. typhosa*. A similar synergism in the use of tyrothricin with sulfonamides against bacteria of the upper respiratory tract was shown by Kelso and Thomson (8).

Sulfonamides and penicillin

Either synergism or additive effects between sulfonamides and penicillin have been reported by many workers. Ungar (9) described a synergistic effect against pneumococci between penicillin and para-aminobenzoic acid or sulfonamides This work was soon paralleled *in vivo* by Soo Hoo and Schnitzer (10) against streptococci. T'sun T'ung (11) reported a similar effect against *Br. abortus*. Bigger (12) in the same year indicated that of several sulfonamides used in combination with penicillin *in vitro*, sulfathiazole was the most effective. This last combination was later found by the same author to exert a synergistic effect against *"B. typhosum."* Vigouroux and Leyton (13) extend the potentiation of penicillin action by para-aminobenzoic acid and sulfonamides against several pathogenic bacteria.

In vitro studies demonstrating the same principle of the increased effectiveness of penicillin in the presence of sulfonamides have been carried out by a number of investigators. Some workers have concluded that, *in vitro*, sulfonamide does not appreciably alter the "bactericidal" action of penicillin if penicillin is already present in amounts above the minimal effective concentration, though sulfonamide does bring about a reduction of this value against several strains of viridans streptococci. The other authors in this group reported that the combined action is one of additive effect, not true potentiation.

Waring and Smith (14) first pointed out the improved therapy in the treatment of pneumococcic meningitis by the simultaneous use of sulfon-

amides and penicillin. A more recent study by Barnet (15) endorsed the value of such a combination against this particular disease. After indicating that either agent alone was repeatedly ineffective, the author stated that the intrathecal use of penicillin G with orally given sulfadiazine provided complete recovery. Zinneman (16) also offered clinical evidence that this combination is substantially more effective than either agent used singly in the treatment of meningitis caused by *H. influenzae*.

The advantage in using penicillin simultaneously with sulfonamides is now being recognized in veterinary practice against *S. agalactiae* and other gram-positive bacteria present in bovine mastitis. Various investigators submitted evidence that penicillin used with a number of sulfonamides is more effective than either agent alone. Johnson and Roberts (17) undertook a critical study to test the synergism concept, and reported that "the infusion treatment of mastitis with the combination of a sulfonamide and penicillin produced better bacteriological and clinical results with fewer ingestions than with either one of the drugs administered separately."

Penicillin and agents other than sulfonamides

Increased effectiveness of penicillin has also been reported when used with other agents, namely with urea, urethane, nicotinamide, ascorbic acid and riboflavin, and with certain dyes. More recently improved results in the treatment of syphilis have been obtained by using penicillin with other drugs. Kolmer (18), in the treatment of experimental syphilis in rabbits, showed that the combination of one-third to one-fourth the curative dose of oxophenarsine reduced the minimum curative dose of penicillin from 100,000 units to less than 10,000 units/kg in acute syphilitic orchitis. Bismuth or potassium tartrate in oil injected intramuscularly also showed "decided synergistic or additive therapeutic effects with penicillin in this disease." Leavitt (19) reported that the use of these same combinations in neurosyphilis provides slightly better results than penicillin alone, but the improvement is not sufficient to warrant the risks of combined therapy as contrasted with the safety of penicillin alone. On the other hand, a positive value in using a combination of penicillin-mapharsen-bismuth in treating 201 cases of early hyman syphilis has been reported. A 5-week treatment provided satisfactory progress in 90 per cent of the cases; 1.5 per cent were failures, and the rest are pending.

The use of caronamide with penicillin, while providing improved therapy, is perhaps beyond the scope of this discussion in that the caronamide is considered to function by reducing the tubular excretion of penicillin and hence prolonging the maintenance of an effective blood level.

The recent work of Pratt and Dufrenoy (20) seems to indicate a true synergism between penicillin and the cobalt ion. The first paper indicated

that trace amounts of cobalt "markedly lowered" the concentration of penicillin required to produce bacteriostasis. Parallel *in vivo* results with respect to the action of this combination against *S. typhosa* in mice are presented in the second paper.

Streptomycin and other agents

Streptomycin is also subject to potentiation by other agents. Thatcher and McLean (5) reported synergism against several gram-negative pathogens between streptomycin, several sulfonamides, and acriflavine and other dyes. The usefulness of a combination in reducing the "survival of adaptive forms that give rise to resistant strains" was pointed out. Similar concepts were independently developed by Klein and Kimmelman (21). These workers concluded that synergism between streptomycin and sulfadiazine was related to delay in the development of resistance to streptomycin brought about by the presence of the sulfonamide. Figures 36 and 37 taken from their publication (21), show that the effectiveness of a given concentration of sulfadiazine is, in general, inversely related to the numbers of cells present in the medium. Hence, it is argued, the "potentiating" effect of the "sulfa" on streptomycin may be due to the fact that the few streptomycin-resistant cells that survive exposure to streptomycin are easily destroyed by relatively low levels of sulfadiazine.

MacLean and Smith (22) have reported the use of a combination of streptomycin and sulfadiazine or sulfathiazole in treatment of nontuberculous urinary tract infections; from this study they concluded that "there was no evidence of increased 'bacteriostatic' action through the addition of sulfa drugs to streptomycin Their conclusions, however, appear to have been based on the complete disappearance of clinical symptoms rather than of the bacteria present at the beginning of therapy, for in only two of twenty-eight cases that received both drugs did the combination fail to remove the bacteria initially present in the urine of the patients, as compared with six of twelve cases receiving streptomycin alone. It seems reasonable to suggest that if symptoms are due to factors other than the specific presence of a particular organism or group of organisms, or if continuous reinfection can occur, then a bacteriostatic agent can not logically be expected to effect an unconditional cure

The value of simultaneous use of streptomycin with sulfonamides seems to be receiving wide acceptance in the treatment of influenzal meningitis Little (23) has reported the experiences of several practitioners to this effect, and his own results have indicated that streptomycin with sulfadiazine is definitely more effective than either agent alone. On the other hand, Alexander and Leidy (24) stated that the therapy value of

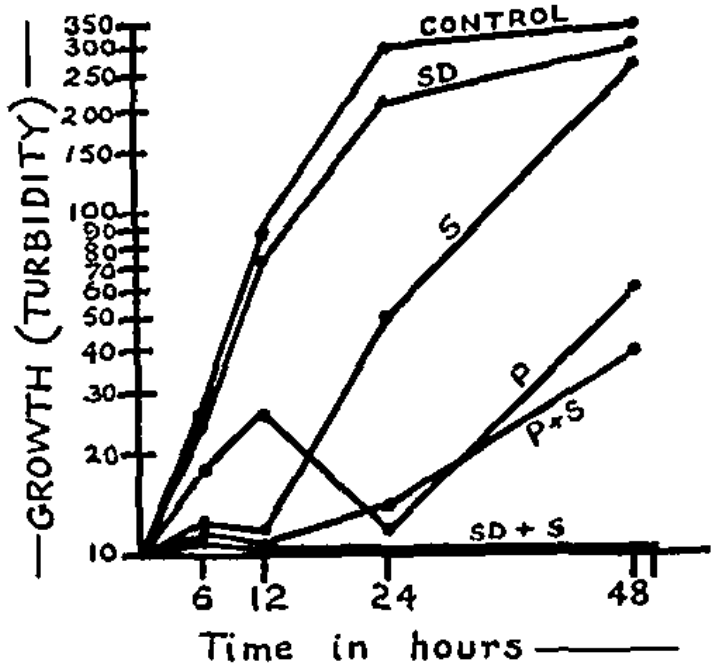
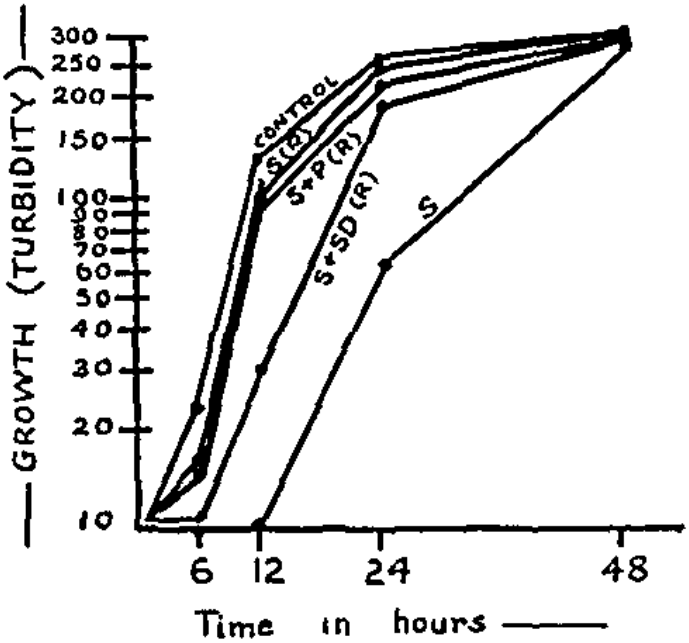


FIG. 36. Inhibitory action of streptomycin, penicillin, and sulfadiazine—singly and combined—on susceptible *S aureus* P = penicillin, 0 06 u/ml. S = streptomycin, 8.0 u/ml. SD = sulfadiazine, 1:5,000. P + S = penicillin, 0.06 u/ml plus streptomycin, 8 0 u/ml (final concentrations). SD + S = sulfadiazine, 1:5,000 plus streptomycin, 8 0 u/ml (final concentrations) (21).



tomycin, 8 0 u/ml seeded with organisms grown for 48 hours in 8 0 u/ml streptomycin plus 1:5,000 sulfadiazine (final concentrations) (21)

streptomycin alone was not enhanced by addition of sulfadiazine against type b *H. influenzae* in mice.

Spink, Hall, Schaffer, and Braude (25) and Pulaski and Amspacher (26) have indicated a combination of streptomycin and sulfadiazine as a specific treatment against human brucellosis. The latter paper indicated that streptomycin alone has no therapeutic value in this disease. This was confirmed by Gilman and LeGrow (27), though contradictory evidence was cited by Spink *et al.* (25). These latter workers noted a valuable correlation between tests for therapeutic agents carried out with living chick embryos and clinical results in human disease. *Br. abortus* was isolated from the liver of embryos treated with sulfadiazine alone in 92 per cent of cases, 77 per cent after streptomycin therapy, but only 13 per cent after use of a combination of the two. In humans the combination of the two was markedly effective, including success in conditions normally proving fatal.

Synergistic combinations are receiving particular study in their application to treatment of tuberculosis. Combined treatment of streptomycin with sulfones has proved of superior value to streptomycin alone. Streptomycin and diasone treatment in experimental tuberculosis of guinea pigs has provided "particularly noteworthy results," according to some investigators. Smith, McClosky, and Emmart (28) have offered similar comment with respect to streptomycin and promin. In experiments with white rats, viable tubercle bacilli were isolated after treatment from all animals receiving promin alone; streptomycin alone reduced the numbers of viable bacteria present, but with the two agents together plates from 41 per cent of tested animals remained sterile. With the last treatment, surviving bacilli were markedly decreased in numbers, with respect to other treatments, and were attenuated.

Smith, McClosky, and Emmart (28) have also compared the action of streptomycin with other sulfones and with sulfadiazine by computing a therapeutic ratio based on the relative amounts of tissue with tubercular involvement in the control animals, compared with that in treated animals (guinea pigs) These results are shown in table 23. According to the authors, these values illustrate a "therapeutic synergy"

McGregor (29) indicated success in treatment of tuberculous meningitis by combined use of streptomycin and promin, streptomycin alone proving a failure in comparable cases.

Morton (30) has pointed out another advantageous field for the use of a synergistic combination of therapeutic drugs. The preoperative use of streptomycin and sulfathalidine in urological surgery has reduced postoperative infection in cases where *E coli*, *Proteus* sp. and *Ps. aeruginosa* had been present Prevention of development of resistance to strepto-

mycin was given as the reason for the advantage in using the two agents together. MacLean, Smith, Bower, and Smith (31) have shown similar value in using streptomycin and sulfathalidine preoperatively in ureterointestinal transplants. "Bowel sterility or nearly so" was accomplished by the combination.

Para-aminosalicylic acid is currently receiving attention as a synergist with streptomycin against *M. tuberculosis*, though workers at the National Institute of Health appear to have developed more promising preparations for this purpose [1] Following Lehmann's indication that para-aminosalicylic acid was the best of many synthetic antibacterial preparations tested in the treatment of tuberculosis, Youmans, Youmans, and Osborne (32), using mice, reported a confirmatory study and showed that para-aminosalicylic acid combined with a daily dose of 750 μg per mouse

TABLE 23

Effect of sulfones and streptomycin alone and in combination on tubercular development in guinea pigs (28)

TREATMENT	RATIOS OF TUBERCULAR INVOLVEMENT OF CONTROLS COMPARED WITH TREATED ANIMALS
Streptomycin alone	14.4
(I) 4-amino-4′-n-propylamino-phenylsulfone (alone)	3.7
(II) 4-amino-4′-n-propylamino-diphenylsulfone (alone)	1.8
(III) Sulfadiazine	1.0
Streptomycin + (I)	28.6
Streptomycin + (II)	18.2
Streptomycin + (III)	14.5

gives a greater effect than either substance alone. This response, however, is only equal to that provided by 3,000 μg of streptomycin alone. From this, the result is considered an additive effect rather than a potentiation. Youmans *et al.* showed later that para-aminosalicylic acid is effective in inverse proportion to the numbers of bacteria present and stated that para-aminosalicylic acid plus streptomycin exert a greater suppressive effect on the tuberculous process in mice than does either substance alone.

Other attempts to find an adjuvant for streptomycin have been undertaken, though no suggestion of the occurrence of a true synergism has been made Slotkin (33) reported that chaulmoogra oil aids streptomycin in its antitubercular effect by rendering the protoplasm of the bacilli more accessible to the bacteriostatic drug. Streptomycin with oil is more effective than when used alone, both *in vitro* and against tuberculosis in guinea pigs.

[1] Data seen on a visit of the AAAS to the National Health Institute, Bethesda, Md.

Studies with a limited number of humans were inconclusive. MacLean *et al.* (31) suggested that the oil may improve the effect of streptomycin in renal and prostatic tuberculosis, though again they indicated inconclusiveness.

Woody and Avery (34) have presented interesting data on the combined effect of potassium iodide and streptomycin on established tuberculosis in guinea pigs KI, the authors reported, may facilitate "solution and adsorption" of caseous matter about the cells, exposing the bacilli and rendering them vulnerable to chemotherapy. With these agents introduced into the guinea pigs 21 days after inoculation, five of ten pigs treated with streptomycin alone showed tubercular involvement of the viscera. No tuberculosis was found in the animals receiving both agents. When treatment was begun 4 weeks after inoculation, the percentage mortality ratios were as follows: controls—100 per cent, streptomycin alone—46.1 per cent, streptomycin plus KI—14.3 per cent.

Anderson and Chin (35) have reported "synergistic" effects of "British anti-lewisite," which is 2,3-dimercaptopropanol, with streptomycin against various *Mycobacterium* species, though the effect of the anti-lewisite was less pronounced than when used with subtilin. Each combination was *ineffective against M lysodeikticus. The mode of action* was not suggested.

The Physiological Basis of Synergism

The foregoing literature indicates clearly that lack of agreement exists as to whether the improved activity of one drug as brought about by the presence of another represents a true synergism or whether merely an additive effect is being observed

Critical *in vitro* studies of the penicillin-sulfonamide interaction have been carried out by a number of investigators. From a study of growth curves, it was indicated that no true potentiation occurs, but that the observed increase in bacteriostasis brought about by the combination is merely an additive effect of the individual components. In agreement with this, others stated that the additive effect occurs only when each agent is present at a *concentration sufficient to initiate bacteriostasis independently*, and that the amount required will depend upon the numbers of organisms present They did not infer that this is the only basis for occurrence of synergism

That "synergism", that is, true potentiation, can occur between drugs is well illustrated by the work of Bliss, who examined the effect of mixtures of insecticides, and of fungicides Bliss (36) recognized three types of joint action, assuming no stable compound is formed through interaction of the ingredients The percentage mortality inflicted was used as

a basis for comparison. The three types are as follows: (a) independent joint action—here joint action is predictable from consideration of the mortality curves of the individual ingredients and the relative susceptibility of the subject to the two poisons; (b) similar joint action, in which the components of a mixture are mutually replaceable, and joint toxicity is predictable on the basis of the relative proportions of each in the mixture; such action would provide the "additive" effect cited in several instances of joint use of bacteriostatic agents; (c) synergistic action, an effect which is subject-specific and is noncomputable from a study of individual effects, though Bliss was able to deduce certain generalizations that are subject to mathematical treatment. All three types may be recorded in the foregoing literature review.

Herman (37), working in the writer's laboratory, has examined the mutual effect of sulfonamides, streptomycin, and penicillin by methods based on those of Dimond and Horsfall, using several species of *Salmonella* as test organisms. To obtain the optimum relative concentration of the two drugs, progressively increasing quantities of one agent and decreasing quantities of the second were added to media containing standard inocula in one series of experiments, and in another series, the second agent was added in ascending amounts and the first in decreasing amounts. Semisolid media were used so that discrete colonies developing from a single cell could be recognized. Effective bacteriostasis was reported when no colonies were visible after incubation at 37°C for 4 days.

Table 21, adapted from Herman, indicates the ratios of the amounts of antibacterial substances required to inhibit growth when used alone compared with the amount required when in a combination. Species specificity is apparent. When combined with a constant sublethal amount of sulfathiazole, neither penicillin nor streptomycin when diluted more than 1:4, is usually effective against the *Salmonella*. Sulfathiazole, however, is effective when diluted beyond 1:8. In this instance the sulfa-streptomycin effect is not ascribable to an additive effect alone. Moreover, sulfathiazole when used in relatively large but sublethal amounts against the several *Salmonella* species, inhibits bacterial motility, production of gas bubbles in the medium, and surface growth The amounts of sulfathiazole which, when combined with penicillin or streptomycin, would completely inhibit growth were so low that if used alone they would provide no evidence of any such macroscopic effect.

Thatcher and Marmur (38) have made preliminary inquiries into the physiological basis of synergism following the working hypotheses elaborated by Thatcher and MacLean (5). These hypotheses were based on the contemporary knowledge of the mode of action of antibacterial agents. It was argued that synergism could be expected according to the following

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vide the full or only explanation. Using actively growing cells of *E. coli*, *Ps. aeruginosa* and *S. aureus* in a complete medium containing lactate as respiration substrate, Thatcher and Marmur have shown that use of a synergistic combination of antibacterial agents brings about a substantial increase in inhibition of oxybiontic respiration as measured by the Warburg method (figs. 38 and 39). This was true for streptomycin with sulfathiazole or euflavin, and for sulfathiazole or sulfanilamide with methylene blue or with euflavin.

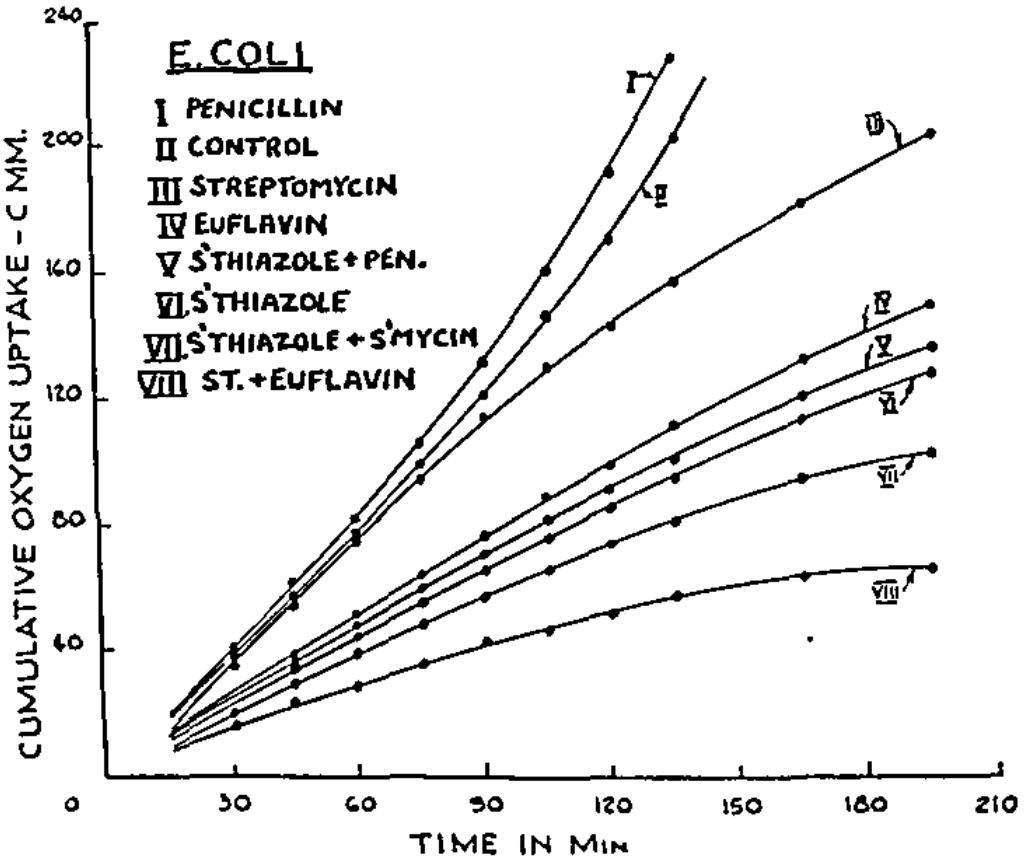

FIG. 38 The effect of antibacterial substances, alone and in combination, upon the cumulative oxygen uptake of active cultures of *E. coli* (38).

These studies suggest that streptomycin and sulfathiazole owe their mutual potentiating effects to the fact that each acts upon a different enzyme sequence The evidence is as follows: (a) Addition of cyanide to streptomycin increases the respiratory inhibition over that of either alone, whereas cyanide has little effect on the inhibition caused by sulfathiazole. This may indicate that cyanide and sulfonamide, each a highly active inhibitor, may under aerobic conditions be primarily active upon the same or mutually dependent systems. Addition of one to the other, therefore, has little effect. On the other hand, streptomycin and sulfathiazole and euflavin (or euflavin and sulfathiazole) inhibit different systems (as shown

concepts: (a) By use of a drug which "blocked" the activity of a respiratory route alternative to that already "blocked" by another bacteriostatic agent. This would lead to frustration of the development of resistance. (b) If the redox potential (Eh) were poised at the site of action of a particular oxidative enzyme, then the action of a specific inhibitor could be increased by poising at an Eh which provided maximum inhibitor-enzyme interaction, or by poising the Eh at a value which interfered with those reactions essential to the synthesis of the metabolite or coenzyme hypothetically being immobilized (for example, sulfanilamide–para-aminobenzoic acid) (c) An additive effect could be expected if two agents were independently active against the same phase of bacterial metabolism. (d) By effecting a significant change in membrane permeability and hence facilitating the development of a critical internal concentration of the antibacterial agent

TABLE 24

*Ratios of amounts of antibacterial substances required to inhibit growth when used alone compared with amount required when used in a combination** (37)

ORGANISM USED	EFFECTIVE RATIOS WHEN USED IN A SULFATHIAZOLE-PENICILLIN MIXTURE		EFFECTIVE RATIOS WHEN USED IN A SULFATHIAZOLE-STREPTOMYCIN MIXTURE	
	Sulfathiazole	Penicillin	Sulfathiazole	Streptomycin
301 *S. enteritidis*	10:1	2:1	10:1	2.5:1
303 *S. enteritidis*	8:1	4:1	8:1	4.0:1
315 *S. enteritidis*	5:1	2:1	5:1	2.5:1
316 *S. enteritidis*	60:1	2:1	6:1	5.0:1
317 *S. newport*	6:1	2:1	6:1	2.5:1
331 *S. thompson*	10:1	10:1	8:1	10.0:1
320 *S gallinarum*	2:1	2:1	20:1	2.0:1
325 *S. pullorum*	4:1	1:1	4:1	5.0:1
308 *S. cholerasuis*	8:1	4:1	8:1	2.0:1
344 *S. cholerasuis*	8:1	1:1	8:1	2.5:1
340 *S. paratyphi*	10:1	4:1	10:1	1.0:1
342 *S schottmülleri*	20:1	2:1	20:1	2.0:1
337 *S typhosa* VII	30:1	4:1	30:1	3.0:1
F D A. *S typhosa*	20:1	5:1	20:1	1.0:1
310 *S. typhimurium*	16:1	4:1	80:1	1.0:1
343 *S typhimurium*	8:1	2:1	40:1	1.0:1

* Incubated at 37°C for 4 days

Admittedly, these concepts were entirely speculative, but some evidence has been obtained in support of more than one of them. It has already been reported that hindrance of the development of resistant strains is part of the basis of synergism, but this would not appear to pro-

is less inhibitory to this organism than to *E. coli*, though the reverse is true for streptomycin and for euflavin. Furthermore, addition of a small amount of pyocyanin (at a dilution of 10^{-7}) to *E. coli* reduces the sulfonamide inhibition. Hence, the cyanide-sensitive systems (including the cytochrome systems) do not appear to be affected by streptomycin or euflavin, whereas part of the sulfonamide effect under aerobic conditions may be against such enzymes or against a system integrated with them. Hence the activity of the two together would result in increased inhibition, as found experimentally.

Quantitatively this would be an additive effect, but it should be borne in mind that a certain minimum of energy over and above that required for cell maintenance is necessary before cells can multiply effectively, and the inhibition of each specifically "attacked" enzyme (not necessarily complete inhibition) could more readily result in a sum total of available energy that is below this minimum. Hence bacteriostasis occurs. This provides a synergism in relation to bacteriostatic effect in that only a small increase in inhibition of energy metabolism over that provided by one agent would be necessary to establish the highly significant difference between tolerance and bacteriostasis.

This is further borne out by the fact that resistance to a synergistic combination develops much more slowly than to either ingredient alone (38). This would be in accord with the concept that resistance to a single agent arises through activation of an alternative system, possibly through modification of an existing enzyme. Thus if the alternative system is being repressed concurrently with the first, then a third system would need to be activated in order for the cell to become resistant to both agents simultaneously. To what degree can this happen?

Similar conclusions have been drawn from studies of the synergism observed between synthetic pterins (compounds of structure similar to "folic acid") and sulfonamides. Sulfonamides prevented synthesis of folic acid (for which para-aminobenzoic acid appears to be a precursor). It was suggested that the inhibitory pterins probably prevent the formation of an enzyme in which folic acid acts as the prosthetic group. Hence, the synergistic antibacterial effect would result from independent inhibition by the respective agents against distinct enzyme systems, just as we have suggested for the streptomycin-sulfonamide interaction. A mutual potentiating effect provided by penicillin and sodium azide or gentian-violet or merthiolate has been explained as due to all agents acting upon a common enzyme.

Eh poising may also be a factor in some instances of synergism. Thatcher and MacLean (5) reported that the mutually potentiating bacteriostasis by sulfonamides and Eh-poising dyes is destroyed by superim-

by their relative response to cyanide). (b) *Ps. aeruginosa* possesses the pigment pyocyanin which can serve as an alternate for cytochrome in the oxidation of α-glycerophosphate to phosphoglyceraldehyde (39). This or-

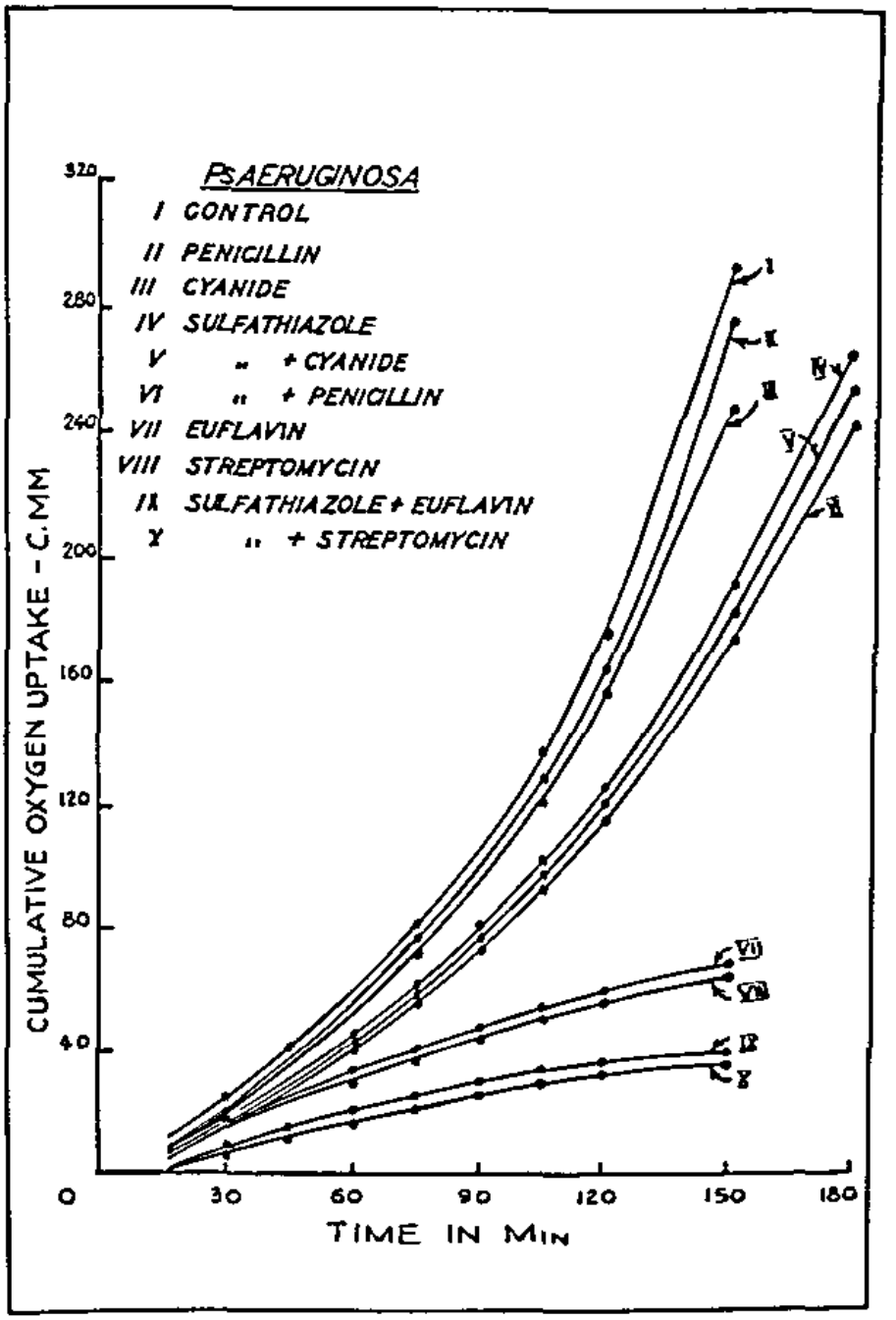

FIG 39. The effect of antibacterial substances, alone and in combination, upon the cumulative oxygen uptake of active cultures of *Ps aeruginosa* (38)

ganism is also relatively resistant to sulfonamides. In accord with this concept of Korr (39), cyanide (at the optimum repressive concentration, namely, 10^{-3} *M*) has very little repressive effect upon the respiration of *Ps. aeruginosa*, while being markedly active against *E coli*. Sulfonamide

brane, particularly by lipoid-insoluble substances, needs to be considered before a full explanation of drug activity is likely to be established.

Thatcher and Marmur examined the effect of salts upon streptomycin and other bacteriostatic drugs from the point of view of their effect on permeability, using monovalent, divalent, and trivalent cations and members of the Hofmeister lyotropic series among the anions. The latter had very little difference in effect, though all salts except carbonates had a repressive effect upon the activity of streptomycin and upon the sulfa–methylene-blue combination. Aluminum chloride was highly inhibitive, much more so than chloride of Na, Mg, or Ca. The aluminum salt at a concentration of 0.0008 *M* was capable of reversing the effect of a normally bacteriostatic level of streptomycin against *E. coli* in nutrient broth. This could be attributed to selective adsorption of the aluminum ions at activity centers of the membrane or intracellularly to reduce, respectively, penetration of streptomycin or its later metabolic interference.

Carbonates had a potentiating effect upon streptomycin and upon sulfathiazole. This effect against streptomycin may be quantitatively reversed by sodium chloride in the respective molar ratio of 1:5. Since sodium chloride greatly reduces adsorption of streptomycin, the carbonate is presumably inactivated by elution, though the direct cause of the carbonate action is not known (it was not due to pH change). Steward and Street (41), however, indicated that in cells of potato tubers relatively high levels of carbonate or bicarbonate at pH 7.0 interfered with the entrance of α-ketoglutarate into the Kreb's cycle from glutamate. The parallelism between the activity of sulfathiazole and triphenyl methane dyes in stopping metabolic use of glutamate, is interesting in relation to a possible mechanism for streptomycin activity, as well as offering an explanation for the enhanced activity of streptomycin by carbonate.

COMMENT

In conclusion, it seems reasonable to say that, under specific circumstances, antibacterial substances including streptomycin may be rendered more effective both *in vitro* and *in vivo* by their use in combination with another appropriate agent. The clinical value of using synergistic combinations would be as follows:

1. A more effective bacteriostatic action is frequently obtained.
2. The danger of development of resistance to a given drug is minimized. This provides opportunity for more prolonged therapy with that drug.
3. An organism having resistance to a specific drug may be destroyed by use of a synergistic mixture when the concentration of the drug to which resistance is manifest is a small fraction of the limiting amount that would be required to destroy the organism with the drug alone.

posing another redox system upon the dye-induced system. Thatcher and Marmur extended their observation to streptomycin and acriflavine, alone or in combination, as well as to sulfathiazole and methylene blue. Addition of ascorbic acid, thioglycollate, cysteine, and potassium ferricyanide at nontoxic levels lowered the potential of the medium in which each of the individual substances or mixtures of streptomycin-sulfonamide, streptomycin-acriflavin, or sulfonamide-methylene blue were present. Inhibition was overcome, and growth occurred normally. The possibility of specific interference by these reducing substances is recognized, but in light of the recent studies (1947) by Pratt and Dufrenoy (20) with regard to the influence of redox potential on penicillin action and on the synergistic penicillin-cobalt reaction, and in view of the influence of Eh in determining selection of hydrogen carriers, the significance of Eh in bacteriostasis and synergism should not be dismissed.

On the other hand, an alternative concept of the effect of "artificial" hydrogen carriers such as the redox dyes seems tenable. If the foregoing evidence is acceptable, that under aerobic conditions sulfonamide can interfere with a cytochrome-linked system, it would appear that sulfonamide can react with more than one system, since it is shown to be as effective anaerobically as aerobically. Hence the particular respiratory pathway against which it is most active under specific conditions might well be dependent upon the Eh of the environment. Thus the reason that sulfonamides are more active in the presence of a concentration of methylene blue sufficient to poise the potential might be that the sulfa drug preferentially inactivates a particular system (methylene blue poises at about the E_{10} value at pH 7.0 for the succinate-fumarate system) and hence diminishes energy output, while at the same time methylene blue further tends to reduce energy available to the cell by itself taking part in the oxidative activities of the cell, but with the resultant free energy being precluded from taking part in the formation of energy-rich phosphate bonds and thus lost to the cell, probably as heat. An additional injurious factor could arise from formation of hydrogen peroxide, though catalase or peroxidase would presumably overcome this.

The suggestions of Thatcher and MacLean (5) with respect to the importance of cell permeability tend to bear more significance in light of recent studies by Gale (10) in that penicillin prevents penetration of glutamic acid whereas sulfathiazole prevents internal syntheses from glutamate.

Strong emphasis has been directed to the need for more finite considerations of permeability modification with respect to the pharmacodynamic action of drugs. Similarly, the possible active role of reversible anion and cation binding as part of a mechanism for permeation of the plasma mem-

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4. Toxicity of a given agent may be reduced, since bacteriostasis may be obtained by using a mixture of drugs at lower concentrations than would be required to provide the same degree of bacteriostasis alone.

5. A longer period of treatment, made feasible by using a suitable combination of drugs, improves opportunity for development of maximum titers of immune bodies.

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CHAPTER 14

ABSORPTION, DISTRIBUTION, AND EXCRETION OF STREPTOMYCIN

The disposition of streptomycin in the mammalian body has been the subject of many investigations both in laboratory experimental animals and in man. In general, the results show good conformity, and a picture emerges which is clear in its main details. Too much emphasis, however, should not be placed on individual sets of data, since the errors inherent in the assay procedures by which such data are collected are large. Rake and Donovick (1) have drawn attention to the wide divergence found in published statements of urinary recovery of streptomycin in man after intramuscular injection—6 per cent to 103 per cent—or after intravenous injection—29 per cent to 92 per cent. Although other factors must enter in, as for example the differences between different patients or test animals (2), much of the variation is undoubtedly due to differences in assay methods and techniques. The warning given in regard to assay values in body fluids in the case of penicillin (3) applies equally well to streptomycin. Values therefore must be considered in light of their actual significance. This will depend on the assay employed, the number of replicates run, the accuracy of the determination of the standard (all assays being in terms of reference units), the types of samples being assayed, and the personnel of the test group. In many cases the error can be expected to be ± 30 per cent, and in not a few it is considerably higher than this. To obtain figures even as good as these (if a biological assay is being used), it is of paramount importance to employ as test organism a bacterium not affected by serum or other body fluids themselves. Finally, in comparing the findings of various authors, it is essential to note whether the streptomycin levels are in terms of whole blood or of serum. Since, as is pointed out below, little if any streptomycin is found to penetrate the erythrocytes, the concentration reported in the serum is usually approximately twice that in the whole blood.

From a physiological point of view it should be pointed out that the disposition of a drug, in this case an antibiotic, depends on its absorption—from the intestinal tract or from the tissues after parenteral administration; on

its partition in the body between cells and extracellular fluid together with its tendency to be bound by plasma proteins; on its being subjected to metabolic transformation within the body; and finally on its method of excretion—whether by the kidneys or by some other route and if by the former whether by active glomerular filtration, with or without reabsorption or secretion by the tubules. Such data as are available in the literature on these points will be presented below

It must be borne in mind that the figures discussed below derive either from normal individuals or from those whose disease does not materially upset the normal processes of disposition. However, in cases of renal impairment due to organic changes, or even as a sequel to the changed metabolism produced by severe illness, both absorption and excretion, but particularly the latter, may be seriously disturbed and entirely different serum levels found.

ABSORPTION

Streptomycin is available as a freely soluble trihydrochloride, calcium chloride double salt, or sulfate and, as such, is usually employed for administration.

Alimentary canal

There is general agreement that absorption of streptomycin from the alimentary canal is poor, or at least erratic, in all mammals. The exact reason for this is not understood, but it is clearly not due to inactivation, since 60 per cent (4) to 110 per cent (5) can be recovered from the feces. Perhaps the most impressive fact concerning absorption from the alimentary canal is this erraticism. The amounts reported to be absorbed vary apparently without regard to the species of recipient or to the exact site of administration and with little regard to the size of dose given Thus, in man, 500,000 units (6) and 600,000 units (7) given by mouth showed no evidence of absorption, no streptomycin being found in the blood or the urine At a dose of 1,000,000 units, some streptomycin usually appeared in the urine (6, 8, 9), but the blood levels varied from none (9) up to 6 units/ml of serum (8). Even at a dose of 4,000,000 units, one observer (10) found only 1 per cent excreted in the urine and none detectable in the blood, whereas another found 0.8 to 1.2 per cent in the urine and only traces in the blood (5). The absorption appears to be better in dogs than in man. Thus, rather high urinary levels were often found at 2,000 to 10,000 units/kg given by mouth (9), and at 200,000 units/kg as much as 10 per cent was present in the urine (4). At best, however, only traces have been found in the blood in this animal (4).

There is little evidence on the manner in which other species, except the mouse, handle streptomycin given by mouth. This rodent, however, apparently absorbs the drug at least as well as the dog, if not better. Thus Stebbins, Graessle, and Robinson (4) noted a blood level of 2 units/ml 45 to 60 minutes after giving 200,000 units/kg by mouth, and Robinson (11) reported that such levels can be obtained by this route as to protect mice against a fatal infection with *S. schottmülleri.* The dose required, however, is thirty times the parenteral dose. In our own laboratory this power of the mouse to absorb greater amounts from the alimentary tract following oral administration has also been noted (12).

The site of absorption from the alimentary tract is of interest. In this connection one should note the comment of Molitor (13) that in man one sees occasionally high and sustained blood levels after rectal administration. No explanation for this is apparent. Our own studies, carried out in both man and mouse (12) suggest that when streptomycin is given by mouth it is absorbed mostly from the upper part of the small intestine. Table 25 shows that there is no immediate rise in urinary levels to suggest absorption from the stomach. On the other hand, the bulk of absorption appears to occur in a relatively brief period, the timing of which differs in individuals but which might be looked upon as an expression of individual variation in gastric emptying time.

Respiratory tract

Streptomycin is commonly administered by inhalation (nebulization). Relatively little has been published, however, on the disposition of the drug following such administration. In general, there appears to be little absorption; high concentrations are found in the sputum, and a marked change in both volume and bacterial flora of sputum occurs (14). Heilman *et al.* noted that inhalation of 500,000 units per day for 28 days produced no demonstrable serum levels and only negligible amounts in the urine (15). In one unexplained instance, however, a patient receiving 500,000 units in 24 hours excreted 30,000 units, or 6 per cent, in the urine over that period. Laurent and her associates (16) found no detectable streptomycin in the serum of rats following inhalation of 2,300 μg (in a mist with 75,000 μg of streptomycin per milliliter). Lung concentrations were high and sustained: 41.6 μg/ml of lung extract immediately and 18.7 μg, 10 μg, and 3 μg respectively after 1, 2, and 3 hours.

Parenteral administration

To ensure adequate serum and tissue fluid levels of streptomycin, parenteral administration must be used As might be expected, most rapid rise of serum levels is seen after intravenous injection and slowest rise after

subcutaneous injection; intramuscular administration gives intermediate results (fig. 40). Species differences are pronounced.

In man, following intravenous inoculation of the sulfate or trihydrochloride the blood level rises immediately to a peak, as shown by serum sample tests. After intramuscular injection, absorption is slower, and peak levels occur 30 minutes (13) to 3 hours (17) later, with a mean time of about 1.5 hours. Subcutaneous injection gives peak levels 45 minutes (13) to 3 hours (8,11) later, with a mean time of about 2 hours. In general, the whole cycle of absorption and excretion is considerably more rapid in the mouse than in

TABLE 25

Urinary excretion of streptomycin hydrochloride following administration per os in a normal subject* (G.R.)

Dose: 1,724,000 units dissolved in 100 ml H_2O

TIME FOLLOWING ADMINISTRATION OF STREPTOMYCIN	VOLUME OF URINE EXCRETED	CONCENTRATION OF STREPTOMYCIN IN URINE	STREPTOMYCIN EXCRETED CUMULATIVE†	CUMULATIVE UNITS EXCRETED / TOTAL EXCRETED
hours	*ml*	*units/ml*	*units*	*per cent*
0	—	<0.6	—	0
½	29	18.4	534	2.7
1½	42	76.2	3,734	19.1
4	69	98.1	10,503	53.7
6	56	68.9	14,341	73.2
8	33	50.1	15,994	81.8
12	91	22.9	18,078	92.4
20½	255	5.1	19,379	99.0
24	153	1.3	19,578	100

* 370 units per mg.

† Total recovery in 24 hours equals 1.14 per cent of the dose administered.

Note that 35 per cent of the streptomycin recovered appeared in the urine between 1.5 and 4 hours. This suggests a peak of absorption between 2 and 3 hours after ingestion.

man following injection by any of these three parenteral routes (1, 4, 9, 11, 18). In monkeys (9,11), dogs (11), rabbits (9), and guinea pigs (9) the situation appears to be intermediate between man and the mouse.

There is less information on absorption following injection of the sulfate or trihydrochloride into a serous cavity, such as the peritoneum, or into the skin, but the general picture would seem to be similar to that after subcutaneous injection (9). The course of absorption from the spinal canal, after intrathecal or intracisternal injection, is completely different. If the meninges are normal and free from inflammation, the so-called blood-brain barrier is a factor, and absorption from the spinal fluid into the blood is as poor as is passage in the reverse direction (13).

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(22) can, indeed, serve as depot substances and do liberate streptomycin slowly (12). In our hands, however, the results obtained with such depot substances in at least two infections, namely, tuberculosis and septicemia with *S. schottmülleri*, have been in no case better than any schedule using the ordinary water-soluble salts of streptomycin and have been actually inferior to some of such schedules.

As might be expected, there is no absorption of streptomycin through the undamaged skin, and, since the cornea of the eye is merely a continuation of the normal skin, it is not surprising that ordinary solutions and ointments of streptomycin produce barely detectable and irregular levels in the aqueous and vitreous humor of the eye (23). Leopold and Nichols (23) were able to show in rabbits that good penetration occurred, however, when the cornea had been abraded or otherwise damaged and furthermore that iontophoresis produced high levels. In a subsequent publication Leopold, Wiley, and Dennis (24) showed that iontophoresis after retrobulbar injection of 200,000 units of streptomycin in 2 ml gave the best vitreous humor levels and that concentrations obtained in this way were much better than those seen after systemic administration. Bellows and Farmer (25) summarized the information on the of absorption into the eye. They showed that with iontophoresis a level as high as 25 μg/ml can be obtained in the aqueous humor of the living eye and 100 μg/ml of streptomycin in the extirpated rabbit eye. They also found appreciable levels after the cornea had been abraded. Finally, they were able to obtain concentrations as high as 50 μg/ml in the aqueous humor by use of corneal baths of streptomycin solutions (50,000 μg/ml) to which aerosol OT had been added. Baths with streptomycin alone produced no penetration. Actually, concentrations greater than 100 μg/ml could be obtained in the aqueous humor but only by use of concentrations of aerosol OT which were locally irritating.

Distribution

Following parenteral administration, streptomycin is as rapidly distributed as though it were present in the extracellular fluids alone (25).

Tissues and tissue fluids

In man (25, 26, 27), as well as in test animals (25, 28), the streptomycin present in the blood is found in the serum, no significant amounts being present in the erythrocytes. Various workers have reported results of assays on extracts of various tissues and have found streptomycin present, but of course this does not mean that the antibiotic was within the cells. We have been unable to find any reference to determination of streptomycin levels in the lymph.

Limited work has been done on depot substances, such as those used, if not abused, in the treatment of certain diseases with penicillin, for example, penicillin in oil and beeswax, or procaine penicillin. Preparations of streptomycin in oil and beeswax and also in solvecillin (a water in oil emulsion) were tested by Kolmer and his associates (19). The results were disappointing. When a dose of 250,000 or 500,000 units of streptomycin in 2 ml of peanut oil with 4 per cent beeswax was administered intramuscularly, only traces up to 2.5 units/ml appeared in the serum and 3.4 to 23 per cent in the urine. The fate of the remainder was undecided. In solvecillin the disposition of the streptomycin was exactly like that of a saline solution of the antibiotic.

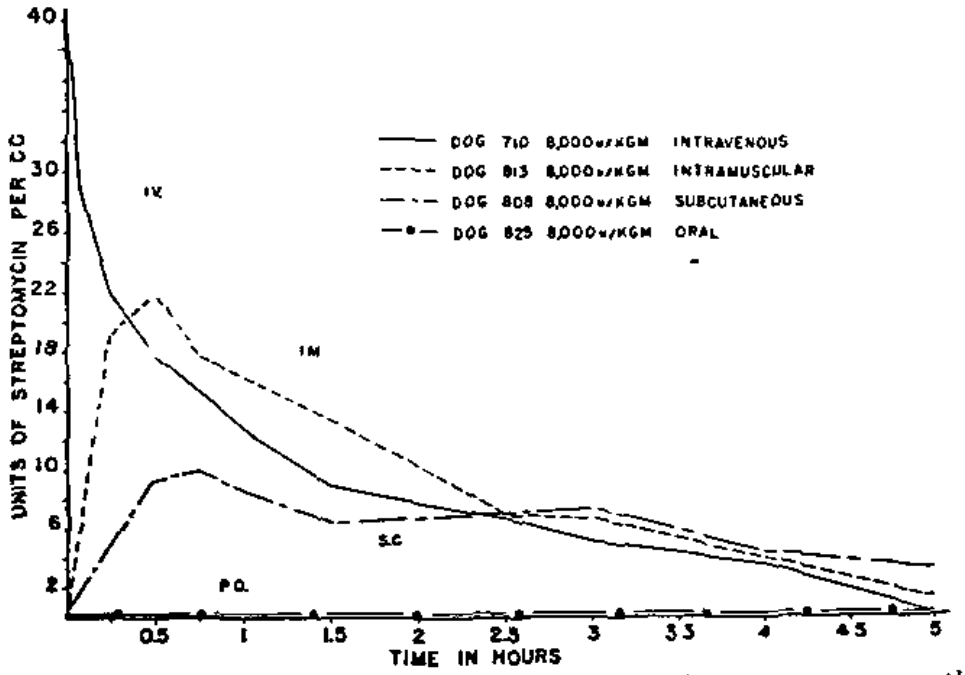

FIG 40. Serum concentration of streptomycin following the various methods of administration (13)

Rake and Donovick (1) reported on the disposition of a streptomycin-alum mixture after Carter, Clark, Dickman, Loo, Skell, and Strong (20) that streptomycin was adsorbed on alkaline alumina. It was found that, at least in the mouse, the streptomycin-alum mixture, although perhaps showing a slightly slower excretion rate, was not sufficiently different from streptomycin sulfate or hydrochloride to be worthy of further consideration In a recent publication, Jadassohn, Cherbuliez, Boymond, and Isler (21) reported the preparation of an insoluble oleate of streptomycin which is active *in vitro* and suggested that it may prove useful in therapy as a depot substance for slow release of the active antibiotic Work on this and other insoluble salts of streptomycin has been in progress in our laboratory for some time. Studies on disposition in the mouse show that insoluble salts

Ocular fluids

As has been pointed out, high concentrations of streptomycin cannot be obtained in the ocular fluids, other than the secondary aqueous humor, after parenteral injection of the drug. Leopold and Nichols (23) studied the penetration of streptomycin into the primary and secondary aqueous humor and the vitreous humor after parenteral administration of the drug in rabbits. Following intravenous injection, streptomycin appeared in the primary aqueous within 5 minutes and was detectable 5 hours later. The concentration increased with the dose of streptomycin. Following intramuscular injection, streptomycin was not found in the primary aqueous before 60 minutes, and it reached its peak in 2 hours. It was still detectable after 5 hours. An increase in dose of streptomycin caused the drug to appear faster in the primary aqueous, and detectable levels were found in ½ hour. A single intravenous or intramuscular injection gave high levels in the

TABLE 26

*Effect of trypan blue on distribution of streptomycin in mice**

HOURS AFTER INJECTION	STREPTOMYCIN PLUS TRYPAN BLUE			STREPTOMYCIN ALONE		
	Blood	Liver	Spleen	Blood	Liver	Spleen
	μg/ml	μg/gm	μg/gm	μg/ml	μg/gm	μg/gm
1	12.0	12.8	59.4	4.0	5.1	0
4	2.5	2.8	6.1	0	0	0
8	0.9	4.2	4.0	0	0	0
25	0.6	6.0	4.9	0	0	0

* From Nelson and associates (29).

secondary aqueous humor. Thus, 10,000 units/kg intravenously gave 20 units/ml in 65 minutes in the secondary aqueous humor as compared to 1 to 3 units/ml in the primary aqueous humor. In the vitreous humor only low levels were obtained. Single intravenous injections of 10,000 units/kg gave no detectable levels in the vitreous, but a single intramuscular injection gave between 1 and 3 units 2 to 5 hours after, and increasing the intravenous dose to 100,000 units/kg gave detectable levels within 5 minutes which persisted for 5 hours These findings were confirmed in three human subjects, two of whom had glaucoma. In these cases 600,000 units of streptomycin administered intravenously gave concentrations of 1 to 3 units/ml in the primary aqueous and 3 to 19 units/ml in the secondary aqueous, when the blood level was between 40 and 75 units/ml.

Serous cavities

Streptomycin passes readily from the blood into the peritoneum, particularly when some pathological process is present. Murphy, Ravdin, and

Adcock and Hettig (26), testing tissues from two patients who had died with tuberculous meningitis while under treatment with streptomycin, found 20 to 95 units of streptomycin per gram of kidney, 6 units/gm of lung, 1 to 5 units/gm of heart muscle, and none in the brain and liver. Komegay, Forgacs, and Henley (9) reported absence of streptomycin in lung, spleen, and liver of guinea pigs receiving 2,500 to 22,500 units/kg parenterally, but they found 5 to 15 units/gm of kidney. Similar results were obtained in mice, rabbits, and monkeys.

Leopold and Nichols (23) reported that, in rabbits, 30 minutes after intramuscular administration of streptomycin (10,000 units/kg) detectable levels were found in the conjunctiva, the extraocular muscle, and the sclera The antibiotic persisted for almost 4 hours. In the chorioretinal layers, the optic nerve, and the cornea, low concentrations did not appear before 2 hours and then only when a dose of 100,000 units/kg was given. The lens showed no streptomycin even after a dose of 100,000 units/kg.

Nelson, Forgacs, and Kucera (29) carried out some interesting studies in mice with the purpose of altering the distribution of streptomycin following parenteral injection. Following the observation that no streptomycin was found in the liver and spleen when levels in the blood were in the therapeutic range, they assumed that therapeutic failure on the part of streptomycin could be explained by inability of the antibiotic to reach intracellularly located organisms. To test this hypothesis, Swiss mice were inoculated intraperitoneally with a single dose of a mixture containing 1,000 units of streptomycin together with 20 mg of trypan blue (an electronegatively charged dye) adjusted to pH 7.4. It had been observed that this mixture would not dialyze through a cellophane membrane, whereas streptomycin dialyzes readily. Control mice received a similar dose of streptomycin in distilled water. Assays were carried out on blood, liver, and spleen at intervals during the next 25 hours, with the results shown in table 26. It appeared that under the test conditions the streptomycin-dye complex was being concentrated in the reticulo-endothelial system, and it was implied that this came about by phagocytosis of the colloidal particles of the mixture.

Postmortem studies in man by Pulaski and Sprinz (30) showed that when death occurred while the patient was under treatment with streptomycin, clinically significant amounts were found in the kidney, liver, muscle, and thyroid, but none in the lymph nodes, spleen, testes, lung, or brain. Only traces were found in the prostate and pancreas. Pus taken from abscesses in four cases was also negative for streptomycin, and Pulaski and Sprinz (30) reported that streptomycin activity is not influenced, except mechanically, by pus. The correctness of this remark may, however, be questioned, since certain factors such as pH certainly do affect the activity of streptomycin (31).

gitis, however, parenteral administration of streptomycin may fail to produce therapeutically significant levels in the cerebrospinal fluid, and intrathecal administration may be necessary (33).

Fetal blood and amniotic fluids

Woltz and Wiley (34) and Heilman, Heilman, Hinshaw, Nichols and Herrell (15) reported streptomycin in the fetal blood and amniotic fluid following parenteral adminstration of the antibiotic to the mother. According to Woltz and Wiley (34), streptomycin was found in the cord blood and amniotic fluid 19 minutes after intravenous inoculation of the mother. The streptomycin concentrations in the cord blood and amniotic fluid were generally lower than in the maternal blood, usually reaching one-half, or less, that of the latter.

Excretion

Urine

The preponderance of excretion of streptomycin is through the kidneys. Broad variations in percentage recovery of streptomycin in the urine of man and test animals have been reported and are commented on earlier in this chapter. On the whole, it would seem that where normal kidney function is involved, 30 to 60 per cent of the streptomycin administered parenterally may be expected to appear in the urine within 24 hours. Urinary excretion is most rapid in the first 2 to 4 hours (7, 26), and the greater part of the antibiotic appears in the urine during the first 12 hours.

Where renal damage is involved, however, the picture may be quite different. Table 27 shows the urinary excretion of streptomycin in a child with grossly defective renal clearance This child at postmortem showed chronic glomerular nephritis, interstitial pyelonephritis and abscess, thrombosis of the small veins of the kidney, and a bacteremia due to *Ps. aeruginosa* (35) The blood level reached a peak within 1 hour following a single parenteral dose and then remained constant, very little streptomycin, that is, less than 2 per cent, appearing in the urine. When additional streptomycin was administered, the blood level rose rapidly and remained at high level for hours and even days.

Because only part of the streptomycin injected parenterally can be found in the urine, and only small amounts have been detected elsewhere, the question of destruction of streptomycin in the body has often been raised. In the case cited above, where less than 2 per cent of the streptomycin administered intramuscularly appeared in the urine, the blood level remained remarkably constant following a single dose, indicating that very little if any of the streptomycin was destroyed. Nevertheless, there appears to be no

Zintel (32) reported levels in the peritoneal fluids equal to, and in most cases higher than, those in the blood, following intramuscular injection in the dog No figures are available on the concentrations under normal conditions in man. Zintel, Flippin, Nichols, Wiley, and Rhoads (8) found that, in a case of ascites, a single intravenous dose of 600,000 units of streptomycin, which produced a blood level of 30 units/ml within 15 minutes, gave rise to the first traces of streptomycin in the ascitic fluid within 0.5 hour. A level of 5 units/ml was found in the fluid in 45 minutes, and this level was maintained through the next 2 hours although the blood level was dropping steadily In another case of ascites, in which streptomycin was administered intramuscularly at a dose of 125,000 units every 3 hours, the blood level at the end of 24 hours of treatment was 15 units/ml, whereas that in the ascitic fluid was 23 units/ml. Buggs, Pilling, Bronstein, and Hirshfeld (6) found levels in ascitic fluid one-third to one-half those in the serum, and Pulaski and Sprinz (30) reported similar findings. As pointed out by Buggs *et al.* (6) in a case of perforated peptic ulcer, in acute peritonitis, streptomycin levels in the peritoneal fluid are exactly equal to those in the serum.

Streptomycin apparently diffuses more slowly into the pleural fluid than into the peritoneal fluid (8). Thus, following adminstration of 600,000 units of streptomycin intravenously, the blood level rose to 30 units/ml within 15 minutes but no streptomycin was found in the pleural cavity. Two hours later, the blood level was 15 units/ml and the pleural fluid contained 6 units/ml. Streptomycin given intramuscularly in intermittent doses of 125,000 units every 3 hours resulted in blood levels of 15 to 20 units/ml and pleural fluid levels of 7 to 18 units/ml. Pulaski and Sprinz (30) reported levels in the pleural fluid of one-fourth to one-half that found in the serum. Buggs *et al.* (6) found that low levels of streptomycin, that is, approximately 1 unit/ml, appeared in thoracic empyemas 3.5 hours after a single intramuscular dose of between 500,000 and 1,000,000 units of streptomycin. Levels of between 2.5 and 3 units/ml were noted in fluid aspirated from the chest of two cases of tuberculosis.

Cerebrospinal fluid

Diffusion of streptomycin into the cerebrospinal fluid does not occur readily in absence of meningitis, and only low and erratic concentrations of streptomycin have been noted following parenteral injection (5,6,7,8,26,27). Remarkably high spinal levels have been reported occasionally in absence of frank meningitis Thus Reimann, Price, and Elias (27) report a spinal fluid level of 20 units/ml in a case of typhoid receiving 4,000,000 units of the drug a day intravenously On the other hand, in the presence of frank meningitis, penetration of the blood-brain barrier by streptomycin has been noted by a number of authors (6,8,15,27) Even in the presence of menin-

gitis, however, parenteral administration of streptomycin may fail to produce therapeutically significant levels in the cerebrospinal fluid, and intrathecal administration may be necessary (33).

Fetal blood and amniotic fluids

Woltz and Wiley (34) and Heilman, Heilman, Hinshaw, Nichols and Herrell (15) reported streptomycin in the fetal blood and amniotic fluid following parenteral adminstration of the antibiotic to the mother. According to Woltz and Wiley (34), streptomycin was found in the cord blood and amniotic fluid 19 minutes after intravenous inoculation of the mother. The streptomycin concentrations in the cord blood and amniotic fluid were generally lower than in the maternal blood, usually reaching one-half, or less, that of the latter.

Excretion

Urine

The preponderance of excretion of streptomycin is through the kidneys Broad variations in percentage recovery of streptomycin in the urine of man and test animals have been reported and are commented on earlier in this chapter On the whole, it would seem that where normal kidney function is involved, 30 to 60 per cent of the streptomycin administered parenterally may be expected to appear in the urine within 24 hours. Urinary excretion is most rapid in the first 2 to 4 hours (7, 26), and the greater part of the antibiotic appears in the urine during the first 12 hours.

Where renal damage is involved, however, the picture may be quite different. Table 27 shows the urinary excretion of streptomycin in a child with grossly defective renal clearance. This child at postmortem showed chronic glomerular nephritis, interstitial pyelonephritis and abscess, thrombosis of the small veins of the kidney, and a bacteremia due to *Ps. aeruginosa* (35). The blood level reached a peak within 1 hour following a single parenteral dose and then remained constant, very little streptomycin, that is, less than 2 per cent, appearing in the urine. When additional streptomycin was administered, the blood level rose rapidly and remained at high level for hours and even days.

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satisfactory answer as yet, other than that of technical factors, for the occasional finding of very low recoveries of streptomycin in the urine following parenteral administration.

Repeated parenteral injections of streptomycin tend to produce an equilibrium between the quantity administered and the amount excreted; as long as renal clearance remains normal, a balance is soon reached and may be maintained indefinitely (fig. 41). Renal damage or, in certain cases, gross physiological disturbances such as are seen in severe infections, particularly in the terminal stages, lead rapidly to rising blood levels, and it

TABLE 27

Excretion of streptomycin in child with grossly defective renal clearance

TOTAL TIME ELAPSED	CUMULATIVE STREPTOMYCIN ADMINISTERED*	BLOOD SERUM LEVEL	CUMULATIVE STREPTOMYCIN EXCRETED	PERCENTAGE EXCRETED
hours	*μg*	*μg/ml*	*μg*	
0	0	0	0	0
—	200,000	—	—	—
0 5	200,000	14.6	0	0
1	200,000	14.6	0	0
2	200,000	11.3	1,370	0.69
3	200,000	11.3	1,727	0.86
4	200,000	11.3	2,668	1.33
7	200,000	13.1	3,619	1.81
22	810,000	27	5,263	0.65
29	1,110,000	40	12,263	1.10
37.5	1,550,000	56.5	16,288	1.05
49.5	2,080,000	76.5	28,048	1.35
69	2,910,000	97	.	..

* At zero hour, 200,000 μg of streptomycin was administered intramuscularly. Seven hours later, administration by continuous drip was instituted.

is essential that the levels of streptomycin reached in the blood be followed in all cases by laboratory assays.

The rate of plasma clearance following parenteral administration of streptomycin has been reported for man and other animal species Adcock and Hettig (26) found that in patients given 50,000 to 250,000 units of streptomycin per hour for 2 hours by means of continuous intravenous infusion, 38 to 67 ml of plasma was cleared of the antibiotic per minute. Although Boxer, Jelinek, Tompsett, DuBois, and Edison (36) did not report the rate of appearance of streptomycin in the urine in their investigation of a chemical method of assaying the antibiotic, they did study the rate of disappearance of the antibiotic from the blood They noted that the rate of disappearance in dogs and in man was exponential after the first 0.5 hour

following a single injection. The rate constant for the decrease of streptomycin concentration was affected by the dose. Thus, in dogs a higher rate constant and smaller volume of distribution were noted at doses between 2,000 and 5,000 μg/kg than at 20,000 to 40,000 μg/kg. The rate constants in man were qualitatively similar to those found in dogs but were somewhat lower in the former, resulting in more prolonged blood levels. The apparent volumes of distribution were considered to be essentially the same for man and dog, that is, approximately 25 per cent as compared to the usually accepted value of 20 per cent for total extracellular fluid. Graham, Van der Brook, and Kuizenga (37) reported that the rates of disappearance of streptomycin from the blood were similar in anesthetized and unanesthetized

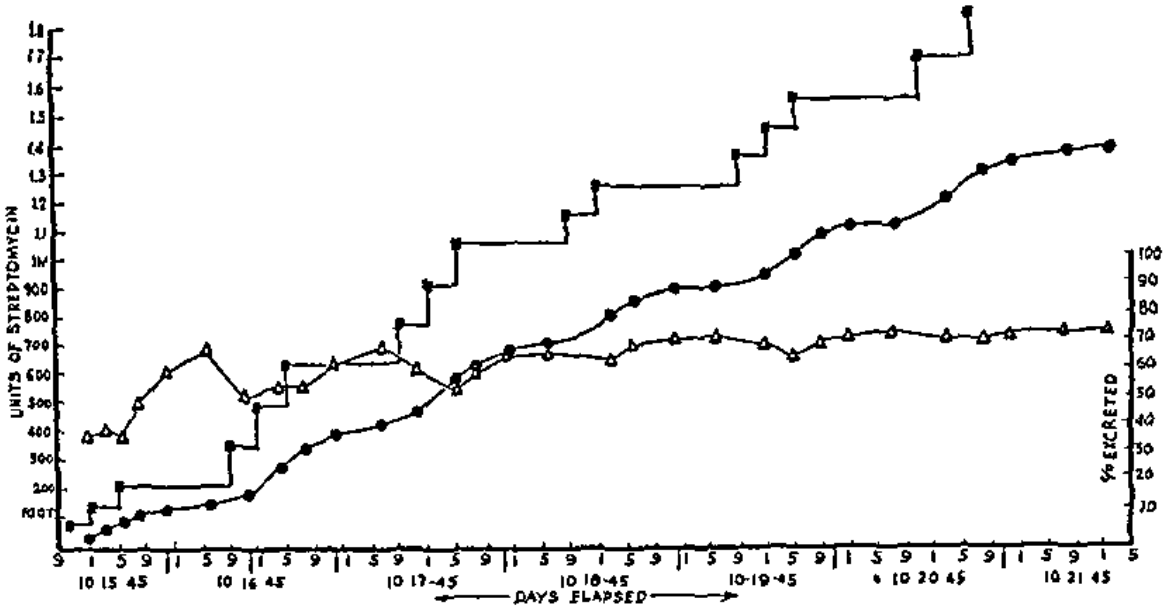

FIG. 41.

■——■

●——●

△——△ per cent excreted.

dogs. Comparison of renal clearance in man and in dogs was also reported by Marshall (25). In the dog the clearance varied from 35 to 59 ml of plasma per minute; in man the volumes were essentially the same. These figures, Marshall pointed out, were lower than values for glomerular clearance and, therefore, unless streptomycin is largely bound by plasma protein, it is excreted by glomerular filtration alone.

Other routes

Although the greater part of the excreted streptomycin appears in the urine, small amounts are excreted by other routes.

Pulaski and Sprinz (30) were unable, however, to find any in the prostatic fluid. Significant amounts do appear in the bile following parenteral injec-

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in the body. No degradation products from such a theoretical breakdown of the antibiotic have yet been demonstrated. Moreover, as pointed out above, in the one case with marked deficient renal clearance, the blood level remained remarkably constant, indicating little if any destruction of streptomycin over this period in this particular case.

It is possible that part of the streptomycin administered may be bound in the body. Direct evidence for this is lacking, but Corper and Cohen (42) have described what they refer to as "a threshold of remote sustained action" of streptomycin in tuberculosis. In their studies these authors treated guinea pigs for 82 to 91 days with 25,000 units of streptomycin daily and then infected the animals with *M. tuberculosis* 1 day after the last treatment with streptomycin. The average survival time of the pretreated animals was 29 days, compared to 22 days of the untreated controls. When pretreatment lasted only 2 weeks, the average survival times of the two groups were not significantly different. From these studies the authors concluded that streptomycin does not act in tuberculosis as it does in the test tube. Lack of detailed description of certain aspects of this work makes interpretation difficult. Thus it was not made clear what variation was found from test to test in average survival of the infected controls, or whether the controls and the pretreated animals were of the same age and weight at the time of their infection But the data presented did not, it would seem, eliminate the possibility that a low therapeutic effect was derived from a reservoir of streptomycin

Dihydrostreptomycin

Only limited work has been done on the disposition of dihydrostreptomycin. Bartz, Controulis, Crooks, and Rebstock (43) reported that dihydrostreptomycin has biological activity qualitatively and quantitatively comparable to that of streptomycin, but did not elucidate further. Donovick and Rake examined the disposition of both crude (44) and pure (45) preparations of dihydrostreptomycin in the mouse. Neither the crude nor the pure dihydrostreptomycin was handled by the mouse in any manner significantly different from that in which this animal handled streptomycin itself (see fig. 42) (45). Hobson, Tompsett, Muschenheim, and McDermott (46) determined the serum concentration of dihydrostreptomycin following intramuscular administration in both man and cat Values comparable to those following intramuscular administration of streptomycin were found in both, although the rate of fall may be slightly slower for dihydrostreptomycin. These authors also determined that dihydrostreptomycin penetrated into the intact central nervous system to a limited degree as did streptomycin, and they found values in the cerebrospinal fluid of 8.1 and 7 5 μg/ml in two patients, neither of whom had any evidence of meningitis.

tion. Zintel *et al.* (8) found that, following a single intravenous dose of 600,000 units, traces of streptomycin were present in the bile within 1 hour, and peak levels as high as 7.5 units/ml were recorded. When 125,000 units of streptomycin was administered intramuscularly every 3 hours for 24 hours the concentration of streptomycin in the bile was 10 units/ml and the total amount excreted was 0.35 per cent of the dose. Adcock and Hettig (26) also noted the presence of 21 units of antibiotic per milliliter of bile following parenteral administration. Gutmann, Trockman, and Ivy (38) reported that in dogs streptomycin is concentrated in the liver. Thus when 187,000 to 375,000 units of streptomycin were injected over a 20-minute period by intravenous drip, maximal concentrations were reached in the hepatic bile in 2 to 3 hours, and as high as 135 units/ml might be obtained. By this time the blood level, which had reached 90 units/ml in 30 minutes, had dropped to between 20 and 30 units/ml. Stebbins, Graessle, and Robinson (4) assayed bile obtained from rabbits during an 8-hour period following a single intravenous dose of 5,000 units of streptomycin per kilogram and found that as much as 10 per cent of the dose administered could be accounted for. Zaslow, Counseller, and Heilman (39,40,41) found that streptomycin was excreted into the hepatic bile of patients who had normal livers. None appeared in the gall bladder, however, when the cystic duct was obstructed. It was therefore concluded that streptomycin reaches the lumen of the gall bladder only via the biliary tree. It was found that the ability to establish antibiotic levels in hepatic bile was a sensitive test for normal liver function, and a minimal level of 1.5 units/ml of hepatic bile within 3 hours after injection of 100,000 units intramuscularly was an indication of such normal function. There was no indication that streptomycin was concentrated in the bile, a conclusion also reached by Pulaski and Sprinz (30), who noted that levels in the bile were only one-fourth those in the serum.

A small amount of streptomycin may be excreted in the feces following parenteral administration. Thus Elias and Durso (10) noted that administration of 4,000,000 units of streptomycin daily by the intravenous route produced 100 to 130 units of streptomycin per gram of feces. Similar results were reported by Reimann, Elias, and Price (5). No figures are available on excretion of streptomycin in the sweat.

These other routes of excretion account for only a very small amount of the streptomycin administered, and the fact remains that, as pointed out above, there is still no satisfactory answer for the occasional very low recoveries of streptomycin in the urine. Some of these low recoveries may be accounted for by technical factors. As it does not seem possible that such an explanation is always the whole story, various workers have implied that some of the streptomycin administered is destroyed or inactivated

In certain cases the serum levels found in man following therapeutic schedules of dihydrostreptomycin given intramuscularly appear to be somewhat greater than those which would have been expected from a similar schedule of streptomycin, but the data are still scanty, and these results by themselves are not sufficiently clear to warrant more than passing comment. Together with the apparent higher urinary excretion of dihydrostreptomycin, however, they offer the possibility that in man less destruction or binding of dihydrostreptomycin than of streptomycin itself occurs, whichever mechanism is responsible for the apparent disappearance of the drug. In any case, attention should be drawn to one important factor in this connection. Unless a dihydrostreptomycin standard is used in assaying the serum levels and unless the figures so obtained are referred to that standard rather than to the ordinary streptomycin standard, the same organism must be used for the serum level determinations as is used for the standardization of streptomycin itself, or at least an organism must be used which has the same sensitivity to both antibiotics. If this is not done, the same type of error as that which occurred with penicillin X will be encountered. This has been discussed elsewhere (3). Obviously, poor choice of test organism could result in figures that would be either too high or too low, depending on whether the organism was more sensitive or less sensitive to dihydrostreptomycin than to streptomycin.

Summary

In general it may be said then that streptomycins are freely absorbed from the tissues, but only poorly and erratically from the gastro-intestinal tract. They appear to be distributed in the body throughout the extracellular fluids and there is no clear evidence that any of them are found intracellularly or that any appreciable protein binding occurs. No clear evidence of metabolism of the antibiotics within the body exists. Finally, they are excreted mostly by the renal glomerular filtration without any tubular reabsorption or secretion.

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Both these patients had received 1 gm of dihydrostreptomycin five times daily for 5 days, the last injection being 14 hours prior to the sampling of cerebrospinal fluid. Levin, Carr, and Heilman (47) also studied the distribution of dihydrostreptomycin in the various body fluids in man. Following intramuscular injection of 1 or 2 gm, the highest concentrations in the blood were found after 1 hour, but significant concentrations were still present at 24 hours. Dihydrostreptomycin passed readily through the placental membrane and was found in the cord blood in concentrations one-tenth to one-half that in the maternal blood taken at the same time. In patients without meningitis, 1 gm of streptomycin administered intramuscularly produced cerebrospinal fluid levels up to 1.5 μg/ml. In the presence

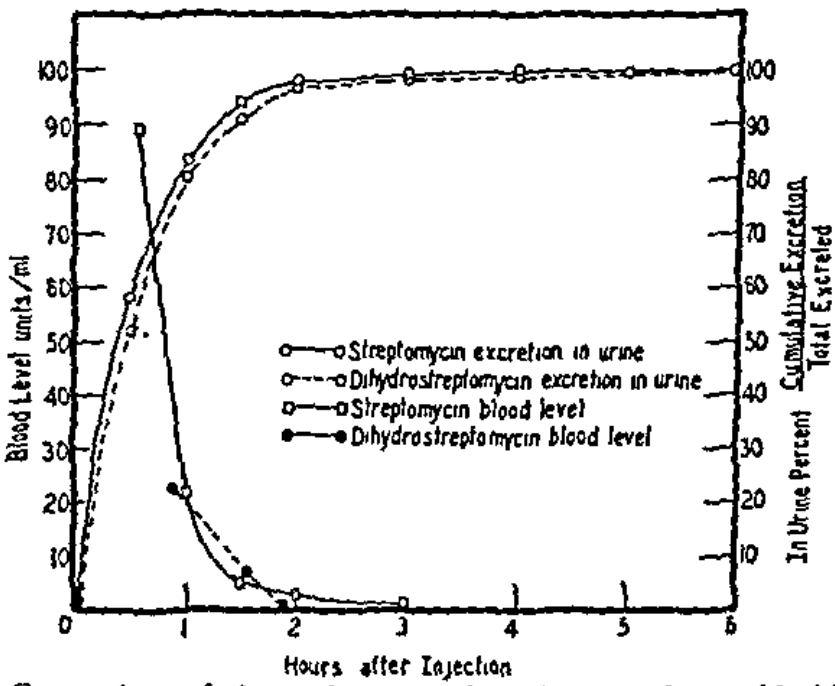

FIG. 42. Comparison of absorption excretion of pure trihydrochlorides of streptomycin and dihydrostreptomycin in mice (45).

of tuberculous meningitis, levels as high as 13.1 μg/ml were seen 2 hours after the last injection of 0.25 gm, a dose that had been given intramuscularly at hourly intervals for 12 hours. At the time the cerebrospinal fluid level of 13.1 μg/ml was found, the blood serum showed a level of 26 μg/ml. Evidence from two cases indicated that dihydrostreptomycin passed freely into the pleural fluid, and levels in this fluid were found to be equal to or greater than that in the blood serum. Dihydrostreptomycin appeared in large quantities in the urine, from 70 to 106 per cent being found in each 24-hour sample. From the limited data on urinary excretion, it would appear that the recoveries of dihydrostreptomycin found by these authors are somewhat higher than those seen with streptomycin itself.

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TABLE 28

*Change of pharmacologic properties of streptomycin with increasing purity**

PURITY (APPROX)	L D. 50 I V	L D 50 S C. AVERAGE	MINIMUM CIRCULATORY EFFECT AVERAGE	RESPIRATORY EFFECTS AVERAGE	PYROGENIC EFFECTS	RENAL EFFECTS	HEPATIC EFFECTS	LOCAL EFFECTS	MINIMAL CENTRAL NERVOUS EFFECTS
	units/20 gm	*units/20 gm*	*units/kg*	*units/kg*					
10%	725 (670–890)	7000	100	5000	+	+	+	+++ at 2000 u/ml	I.C. at 500 u/kg S.C 13 days at 50,000 u/kg
25%	2200 (1750–2600)	10,000	900	15,000	±	+	+	+++ at 20,000 u/ml	I.C. at 1100 u/kg
50%	2500 (2000–2920)	10,000	4000	25,000	−	+	+	+ at 133,000 u/ml	I.C. at 1700 u/kg S C 32 days at 50,000 u/kg
95% to 100%	4000 (3500–4640)	25,000	25,000	None at 40,000	−	−	−	None at 133,000 u/ml	I C at 2500 u/kg S.C 43 Days at 50,000 u/kg

* The L.D. 50 studies were conducted in mice, circulatory and respiratory effects were studied in cats, pyrogenic effects, central nervous effects and local effects in rabbits, and renal and hepatic effects in dogs and monkeys.

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CHAPTER 15

THE PHARMACOLOGY OF STREPTOMYCIN

Among the antibacterial agents, and indeed among drugs generally, streptomycin occupies a distinguished place because of its high chemotherapeutic activity and its relatively low toxicity. Although the safe therapeutic range of this antibiotic is exceeded by that of penicillin, streptomycin nevertheless is far less toxic than most other chemotherapeutic agents such as sulfonamides, arsenicals, quinine, mercurials, and azo dyes. It is one of the safest drugs known. No deaths attributable to the administration of pure streptomycin by the usual parenteral route have been reported; and the neurotoxic side reactions that occur after prolonged administration of large doses do not imperil the life of the patient, although they constitute a major obstacle to long-continued therapy and may seriously incapacitate the patient.

To understand certain contradictory statements on the pharmacological properties of streptomycin made by different investigators, or indeed by the same group of investigators at different times, it is necessary to keep in mind that streptomycin is not prepared by chemical synthesis but is obtained by extraction from bacterial culture media and subsequent purification. Only 5 years have passed since its discovery was first announced, and during this short time the purity of commercial lots has been increased from the initial 10 to 15 per cent to the present value of more than 98 per cent and, as a consequence, the material used during this period for pharmacological and clinical investigations has varied greatly in purity (table 28). Finally, the methods of determining the potency of streptomycin assure the user only of a definite chemotherapeutic activity but do not indicate the extent to which impurities or degradation products may be present.

The present standards of the Food and Drug Administration require, in addition to certain chemical and physical specifications, relative freedom from pyrogens and impurities of histamine-like character; and a minimal potency of 400 mg of streptomycin base per gram of solids. However, since pure streptomycin salts contain approximately 800 mg of streptomycin base per gram of solids, it follows that samples of a purity of 50 per cent still may pass the official tests. Since the weight of streptomycin is not indicated on

characteristic toxic property of streptomycin, namely, its selective neurotoxic effect on the eighth nerve, affecting the vestibular and auditory functions, may vary considerably with the purity of the material (2) (fig. 43). It is therefore understandable that contradictory statements regarding the pharmacological and toxicological properties of streptomycin have been made. The now general availability of much purer preparations, however, has minimized lot-to-lot variations and discrepancies and should result in a better agreement of the results of pharmacologic and clinical investigations

In the following description of the pharmacological properties of streptomycin, are presented data (some not previously published) which were obtained with essentially chemically pure material; in addition, reference is made to the results obtained with the earlier and less pure products, not only to complete the picture but also because streptomycin of less than 95 per cent purity will still be used clinically for some time to come. Pharmacological and toxicological properties that have mainly academic interest and affect the practical use only to a minor degree are treated briefly.

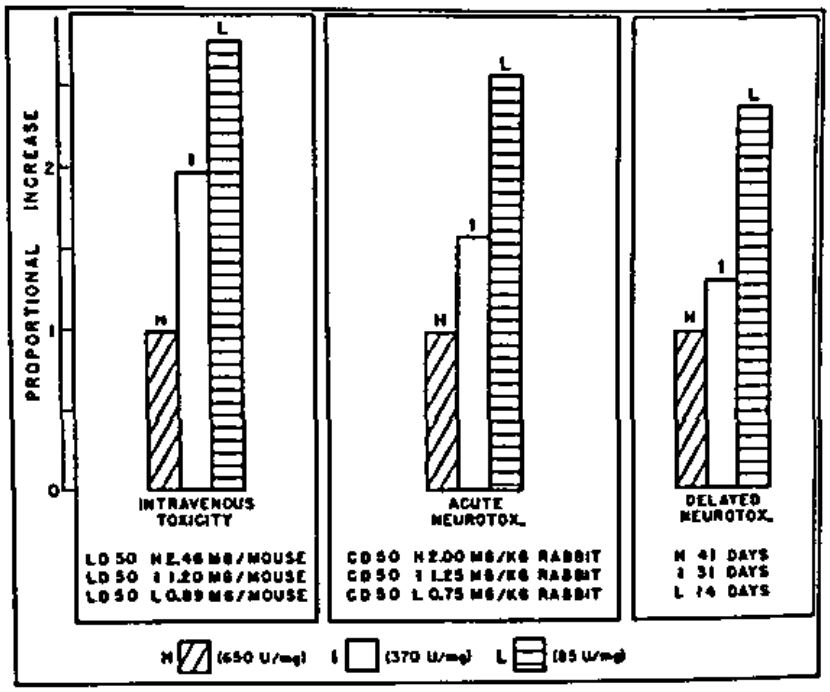
Acute Toxicity

Streptomycin produces definite toxic effects when administered parenterally, although the doses required are far in excess of those needed to obtain chemotherapeutic results. As might be expected, acute toxic effects are most pronounced with intravenous and intracisternal injection and least with oral administration; the intramuscular and subcutaneous routes occupy an intermediate place (1). The intracisternal injection results in acute neurotoxic manifestations such as clonic-tonic convulsions (3, 4) which are not observed with other modes of administration; they are described in detail later in this chapter. The intravenous injection of streptomycin causes death by respiratory paralysis, this mode of administration is used in one of the standard "safety" tests prescribed by the Food and Drug Administration, since it gives results which are easily reproducible and which lend themselves readily to standardization, particularly if proper precautions have been taken regarding speed of injection; concentration of solution; weight, sex, and strain of mice; and, in general, maintenance of uniform test conditions

Following intravenous injection of a lethal dose of streptomycin, death usually occurs within 5 minutes and very seldom is delayed for more than 30 minutes. Ott (5), who conducted an exhaustive statistical study on the basis of more than 2,000 individual experiments, found that of 10,000 mice surviving the initial 30 minutes, only 10 died during the following 2 days. No deaths occurred among 2,500 surviving mice that were observed for 10 days after injection. The fact that animals which survived the initial shock of the intravenous or subcutaneous injection of large doses remained alive

the label (the only figure given being that of a streptomycin base equivalence), the user has no immediate information on the actual purity of any particular lot unless he weighs the contents of the ampoule or directs an inquiry to the manufacturer.

Unfortunately, pharmacologists and clinical investigators frequently fail to realize that the words *1 gram* or *5 gram*, for example, printed in bold type on the label, refer to antibacterial activity equivalent to that of 1 or 5 gm of streptomycin *base*, but not to the actual quantity of material present in the container.



higher purity (700–800 units/mg), with intravenous toxicity ranging from 3000–4000 units/mg.

The varying amounts and types of impurities present in streptomycin lots of intermediate purity, as well as the possible formation therein of degradation products and isomers, have made it difficult to obtain the true picture of the pharmacodynamic properties of pure streptomycin. On the whole, the continued purification of the product has eliminated not only those toxic effects which even in the early days were attributed to impurities, but also some of those which erroneously were regarded as intrinsic properties of the streptomycin molecule itself, for example, the reno- and hepato-toxic action (1). Furthermore, there is now ample evidence that even the most

5 seconds. Constant improvement of the material made it possible to raise these requirements, so that today the minimum required dose is 1,000 μg of streptomycin base, injected in the same manner.

The question of the significance of intravenous toxicity tests has frequently been raised, and much effort has been spent to discover a relationship between the acute toxicity in mice and the degree of tolerance in man. The early studies had proved that there is no constant relationship between the quantity of streptomycin (base) administered and the number of deaths resulting therefrom (fig. 44). This suggested that the acute toxicity depended not only upon the dose of streptomycin itself, but also upon the

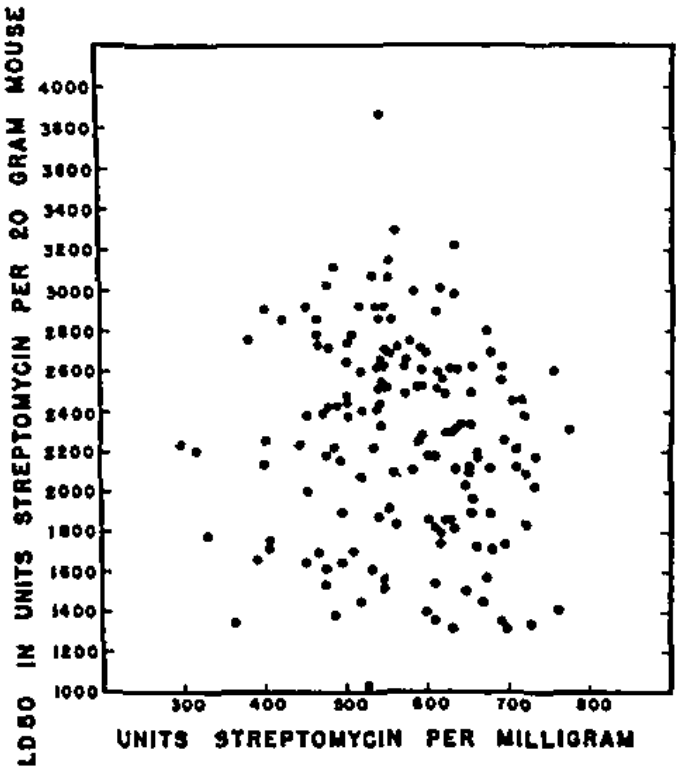


Fig. 44. Acute subcutaneous toxicity in mice of 200 lots of streptomycin

quantity and type of contaminants. All attempts, however, to correlate the acute toxicity of individual streptomycin lots in animals with their general clinical tolerance have failed. Nevertheless, this "safety" test seems to be justified because experience has shown that even seemingly slight variations in the streptomycin broth or in the extraction and purification processes may be reflected in the outcome of acute intravenous toxicity tests. Furthermore, though there is no strict parallelism between the intravenous toxicity and other pharmacologic effects, such as neurotoxicity, sufficient data have been accumulated (8) to indicate that lots which possess a high intravenous toxicity in animals often are also less well tolerated by patients. Thus, although a higher intravenous toxicity is not indicative of any one specific clinical side reaction, it does suggest that the lot in

is in sharp contrast to the results obtained with streptothricin, an antibiotic otherwise closely related to streptomycin (6).

The signs of acute streptomycin poisoning, following a subcutaneous or intravenous injection, consist of restlessness, labored respiration, loss of consciousness, and coma (1). In addition to these signs, larger animals such as cats and dogs may exhibit nausea, vomiting, and ataxia. In all warm-blooded species death occurs apparently through respiratory failure, since the heart continues to beat for several minutes after respiration has ceased. Prompt initiation and maintenance of artificial respiration may often prevent an otherwise fatal outcome. Frogs, which do not depend upon pulmonary respiration, survive many-times fatal doses, provided they are kept partly submerged for several days in tanks where the water is changed frequently.

TABLE 29

Average acute toxicity of streptomycin calcium chloride complex

L.D. 50 in mg of base/kg

SPECIES	INTRAVENOUS	SUBCUTANEOUS	PERORAL
Frog		>1000	
Mouse	200	>700	9000
Rat	175	>600	>6000
Guinea pig		>600	
Rabbit	225	>600	
Cat	150	600	>2000
Dog		>300	
Monkey		>400	

The sensitivity of different animal species to single large doses of streptomycin varies considerably; frogs appear to be least sensitive, monkeys and dogs most sensitive, with cats, rabbits, guinea pigs, rats and mice occupying an intermediate range (table 29). The acute toxicity for man is not known; but single doses as large as 1 gm and total daily doses up to 10 gm have been given by intramuscular injection without untoward signs (7).

It must be pointed out that the above data refer to the pure streptomycin preparations available today, earlier experiments conducted with less pure samples showed not only wide variations among individual batches but also a higher degree of toxicity.

As was mentioned previously, the determination of intravenous toxicity is one of the official Food and Drug Administration tests which each lot of streptomycin must meet before it can be released for clinical use. The minimum L.D. 0 which a sample has to pass was originally set at 300 μg of streptomycin base per kilogram, injected in 0.5 cc of water or saline within

itself, but to an impurity of histamine-like nature, possibly histamine itself. Since the present Food and Drug Administration standards require a relatively high degree of freedom from that impurity, this circulatory side reaction is no longer being observed.

Even pure streptomycin in large doses causes a certain gradual fall of arterial blood pressure; this, however, is altogether different from the steep drop found in a histamine-contaminated sample. Very large doses, such as 200 to 400 mg/kg usually produce irreversible effects. The blood pressure falls to as low as 10 to 15 mm Hg and, provided artificial respiration is maintained, remains at this level until the excess streptomycin has been excreted. During this stage of respiratory and vasomotor paralysis, the heart continues to beat regularly. If death occurs despite artificial respiration, it is probably caused by paralysis of the vasomotor centers, which then also fail to respond to such stimuli as increased carbon dioxide tension, picrotoxin, or metrazol (1). Electrocardiograms taken at frequent intervals during and after injection of normal to very large doses of streptomycin show no significant changes.

Respiratory Effects

Small doses of pure streptomycin (in the order of 0.1 to 0.2 mg/kg) increase both the frequency and the amplitude of respiration, whereas doses of 10 to 100 times that magnitude, particularly when injected intravenously, cause respiratory depression. This is one of the first signs of acute poisoning from streptomycin and is, as has been pointed out previously, the cause of death in acute toxicity experiments.

Renal and Hepatic Effects

The earlier preparations of streptomycin definitely affected the renal and hepatic function of animals and man In addition to a marked and prolonged reduction of water diuresis, which could be ascribed to the histamine-like contaminant, there were observed proteinurea, increase in blood urea values, occasional hyaline and granular casts, and blood cells in the urine even if histamine-free material was used, particularly when given repeatedly and in larger doses (200 to 300 mg/kg) (1, 9, 11, 13, 14).

Animals treated in this manner showed at autopsy albuminous detritus in the subcapsular spaces of the kidney, fatty metamorphosis, and occasional tubular necrosis. It was generally assumed that this reno-toxic effect was an intrinsic property of streptomycin. Repetition of such experiments in dogs and monkeys with the presently available pure streptomycin (10, 12) raises considerable doubt, however, of the validity of this assumption, since the animals injected daily for 2 and 3 weeks with 200 and 300 mg of the pure material failed to develop any of the functional and his-

question differs from the standard and should be more completely investigated.

Chronic Toxicity

Although, as has been pointed out, acute toxicity experiments are justified primarily as an additional control in large-scale production, chronic toxicity studies are of much greater interest because they approach more closely the conditions of actual clinical use. Figure 5 summarizes investigations of this nature performed in the same laboratory (Merck Institute) under identical experimental conditions.

Doses up to 100 mg/kg of impure streptomycin hydrochloride, injected subcutaneously, were well tolerated by rats for several weeks; and up to 300 mg/kg, mixed with the diet, could be fed without significant effects upon the general health and the growth rate. Dogs and monkeys, given subcutaneously 100 to 200 mg/kg of a preparation of intermediate purity developed signs of toxicity consisting of loss of appetite and occasional tenderness and sores at the site of injection. These animals also exhibited proteinuria and appearance of casts in the urine (9). Similar experiments, conducted more recently, however, with up to 300 mg/kg of pure streptomycin (10), failed to elicit such changes, and biochemical tests of renal and hepatic function, as well as microscopic examination of the urine, failed to reveal any of the changes previously reported. The conclusion seems justified, therefore, that the toxic effects previously described were due to impurities. Although the reno- and hepato-toxic effects were not observed when pure streptomycin was used, there were essentially no changes in the neurotoxic signs. The latter must therefore be regarded as an intrinsic property of streptomycin.

The pathological findings after repeated injection of earlier lots of streptomycin in experimental animals consisted of necrotic or inflamed areas at the site of injection, as well as fatty metamorphosis of the liver and, less frequently, of the kidney (11). Experiments in monkeys indicated that these changes were reversible, provided drug administration had been discontinued before irreparable anatomical damage had been done. A recent repetition of these experiments with pure streptomycin failed to produce such changes (12).

Circulatory Effects

Early lots of streptomycin frequently elicited pronounced circulatory effects, such as a steep fall of arterial blood pressure and peripheral vasodilatation. In man, such effects resulted in flushing of the ears and the facial and abdominal skin, attacks of dizziness, and, occasionally, fainting spells (13). This circulatory action, however, was due not to streptomycin

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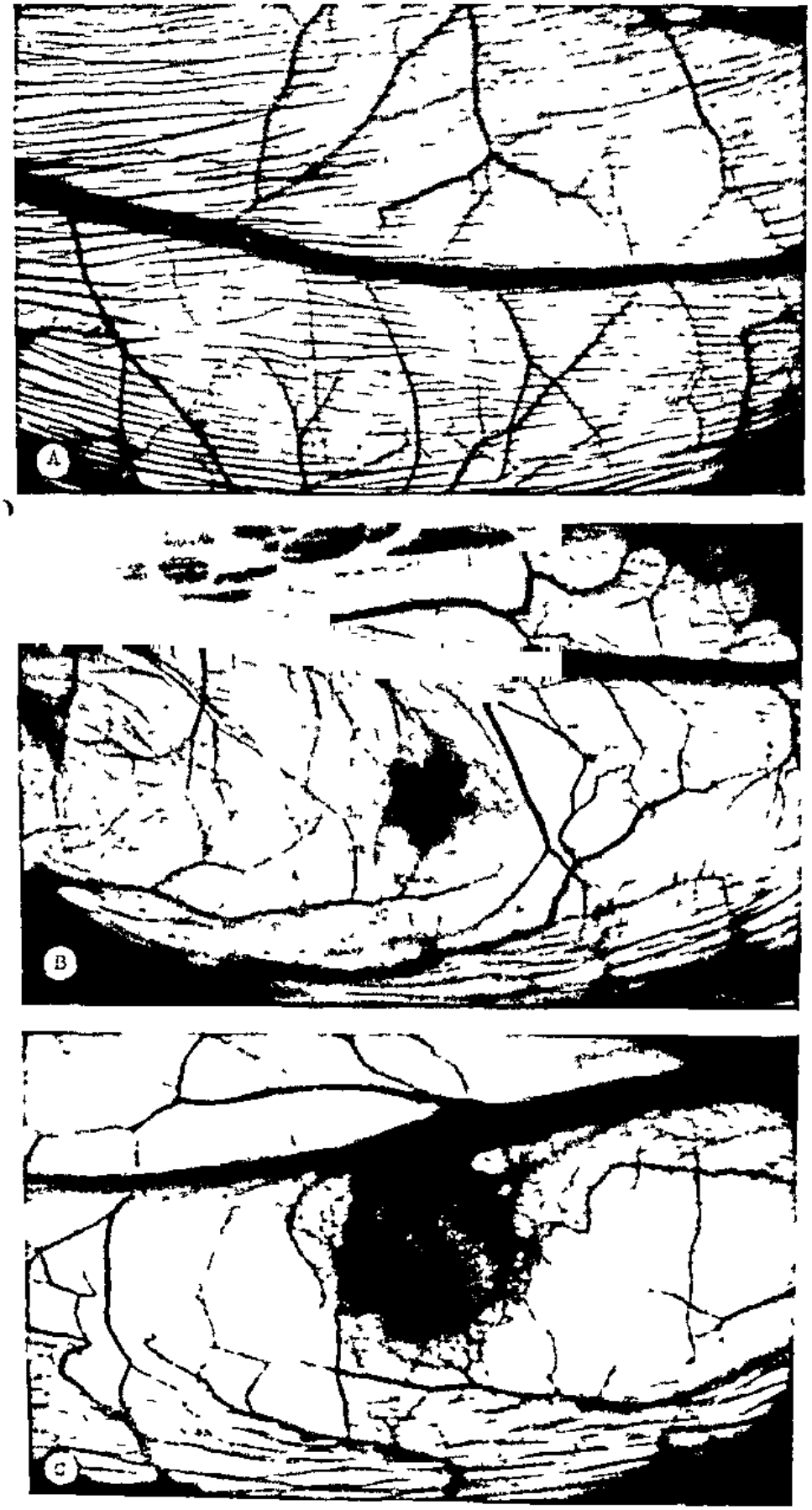

FIG. 45. [illegible] a 20 per cent sol[illegible]cium chloride co[illegible]

tologic changes observed with the older, although highly purified and histamine-free preparations.

The hepatic effects following injection of the older type of streptomycin similarly failed to develop when chemically pure streptomycin was used. The conclusion seems justified therefore that, in animals, pure streptomycin is essentially free from reno-toxic and hepato-toxic effects.

Miscellaneous Pharmacodynamic Properties

Smooth muscle

Streptomycin produces a slight relaxation of the isolated uterus and intestine when it is added to the organ bath in a concentration of 25 to 50 mg/100.

TABLE 30

Effects of daily repeated administration of streptomycin in various animal species

SPECIES	GRAMS STREPTOMYCIN BASE PER KG BODY WEIGHT	MODE OF ADMINISTRATION	AVERAGE NUMBER OF DAYS TO OCCURRENCE OF TOXIC SIGNS	REMARKS
Mouse	2 × 0 4	S C.	28+	Neurotoxic signs
Mouse	1.2	P O.	30	No reaction
Rat (adult)	2 × 0 4	S C.	34	Neurotoxic signs
Rat (weanling)	0 8	P.O.	42	Hyperexcitability
Guinea pigs	2 × 0 4	S C.	14	L.D. 50
Rabbit	3 × 0 3	S.C	10	Neurotoxic signs
Cat	0.4	S.C.	6	Neurotoxic signs
Dog	0 2	S.C.	18	Neurotoxic signs
Monkey	0 2	S.C.	5	No reaction

The streptomycin used in these studies contained from 700 to 730 units/mg

Gastric secretion

Streptomycin lots containing the histamine-like impurity greatly stimulate the secretion of gastric juice and free hydrochloric acid (15). Pure streptomycin, not only is free from these effects but actually depresses the stimulating action of histamine on the secretion of hydrochloric acid. Streptomycin, although partly excreted through the bile and the pancreatic juice, does not significantly alter the secretory activity of these organs.

Local effects

As might be expected, the local effects of streptomycin vary greatly with the purity of the preparation as well as the concentration of the solution. The lower the purity, or the higher the concentration, the greater is the

flora of the intestinal tract and thus might affect blood coagulation indirectly. Smith and Robinson (23) have shown that the addition of streptomycin to the diet of mice in amounts equivalent to a daily intake of 30 to 300 mg/kg of streptomycin base reduced within 24 hours the coliform count from a normal of approximately 100,000 bacteria to one of 100 bacteria per 3 mg of feces and that this low level was maintained throughout the period of treatment. A significant reduction also occurred in the number of nonlactose fermenting organisms. Although these authors did not observe the development of an overt vitamin deficiency in these animals fed a nutritionally well balanced diet, Emerson and Smith (24) found that rats on a purified diet containing streptomycin and providing a daily intake of 580 to 875 mg of streptomycin base per kilogram, developed signs similar to those observed in biotin deficiency. Changes in prothrombin time were not noticed. This is in contrast to findings of Ravdin *et al.* (25) that patients given streptomycin by mouth develop within 4 to 5 days a definite prolongation of prothrombin time, which returns to normal after discontinuation of streptomycin therapy and can be counteracted by administration of vitamin K or by blood transfusion.

Neurotoxic properties

The neurotoxic properties of streptomycin, first described by Hinshaw and Feldman (26) in man and by Molitor *et al.* in animals (1), consist of a selective action on the vestibular and auditory mechanism. They are the most characteristic and serious toxic manifestations of this antibiotic and must be regarded as an intrinsic property of the streptomycin molecule, since they develop upon prolonged administration of large quantities of even the purest material. The early clinical observations indicated, however, that the amount of drug and the length of time required to produce the first signs of neurotoxicity varied considerably with the purity of the streptomycin used. A systematic study in animals (2) not only fully confirmed these clinical observations, but clearly indicated a close inverse relationship of purity and neurotoxic potency (fig. 46).

In view of the high selectivity of the neurotropic effect of streptomycin, it appeared probable that the factor responsible for variations in the neurotoxicity of certain lots of intermediate purity was not an accidental impurity but rather was chemically related to streptomycin. Though the exact nature of this factor—or factors—has not yet been fully determined, it has been shown in animals (2) that streptidine produces neurotoxic signs which are virtually indistinguishable from those of streptomycin. Since streptidine is practically devoid of the antibiotic effect of streptomycin and is not detectable by the chemical tests routinely employed for determination of streptomycin, its presence in the form of a degradation product

probability of local reactions. Pure streptomycin is relatively free from such irritating effects; it may cause pain, however, if injected subcutaneously or intramuscularly in concentrations exceeding 20 per cent. No signs of local damage were found in the veins of rabbits or dogs after repeated intravenous injection of streptomycin solutions containing up to 75 mg/ml (1).

The relative degree of irritation can be assayed by subcutaneous injection of 0.05 ml of 30 and 40 per cent solutions of streptomycin, respectively, into a rabbit's ear and observation of the vascular reaction by transillumination (fig. 45). Clinically well tolerated lots do not produce an appreciable degree of irritation during a 2-day observation period (16).

The effect of streptomycin on the migration of macrophages and the growth of fibroblasts has been reported by Heilman (17) and Bucher (18). Both authors found streptomycin to have a very low degree of cytotoxicity, though exceeding that of pure penicillin G, it was approximately only one-fifth that of one of the sulfones (Cilag) and one twenty-fifth that of paraaminosalicylic acid. Howes (19) has investigated the effect of streptomycin on wound healing in experimental animals as well as in patients. Although epithelialization was retarded by concentrations exceeding 0.4 per cent, it was not adversely affected by a concentration of 0.2 per cent. This lower concentration, particularly when used in combination with a sulfonamide, proved to be of definite clinical value by promoting the healing of contaminated wounds. Experiments performed in the Merck Institute with streptomycin of presumably much greater purity not only confirmed these results but indicated an accelerated rate of wound healing, when a concentration of 0.75 per cent of pure streptomycin in agar base was used; a concentration of 1 per cent failed to interfere with the growth of healthy granulations, but did not accelerate the wound healing (20).

Effect on blood coagulation

The reports on the effect of streptomycin on the coagulation time of blood are contradictory. Macht (21) reported that "next to the chemotherapeutic properties of penicillin and streptomycin and their low toxicity, the most important pharmacologic finding is their thromboplastic activity . . ." Overman and Wright (22), on the other hand, reported that streptomycin prolongs the prothrombin time and the coagulation time of blood.

Since no other reports have appeared in the literature, in spite of the very wide use of streptomycin, it would seem justifiable to assume that, regardless of what action streptomycin might exert on blood coagulation time, the effects are not sufficiently marked to cause clinical complications and difficulties. The possibility exists, however, that prolonged oral administration might interfere with the vitamin K synthesis by the bacterial

pigs, rats, mice, pigeons, chickens), cats appear to be the most sensitive, a daily dose of 50 to 150 mg/kg producing definite neurotoxic signs within an average of 20 days. These signs consist of changes in gait and posture;

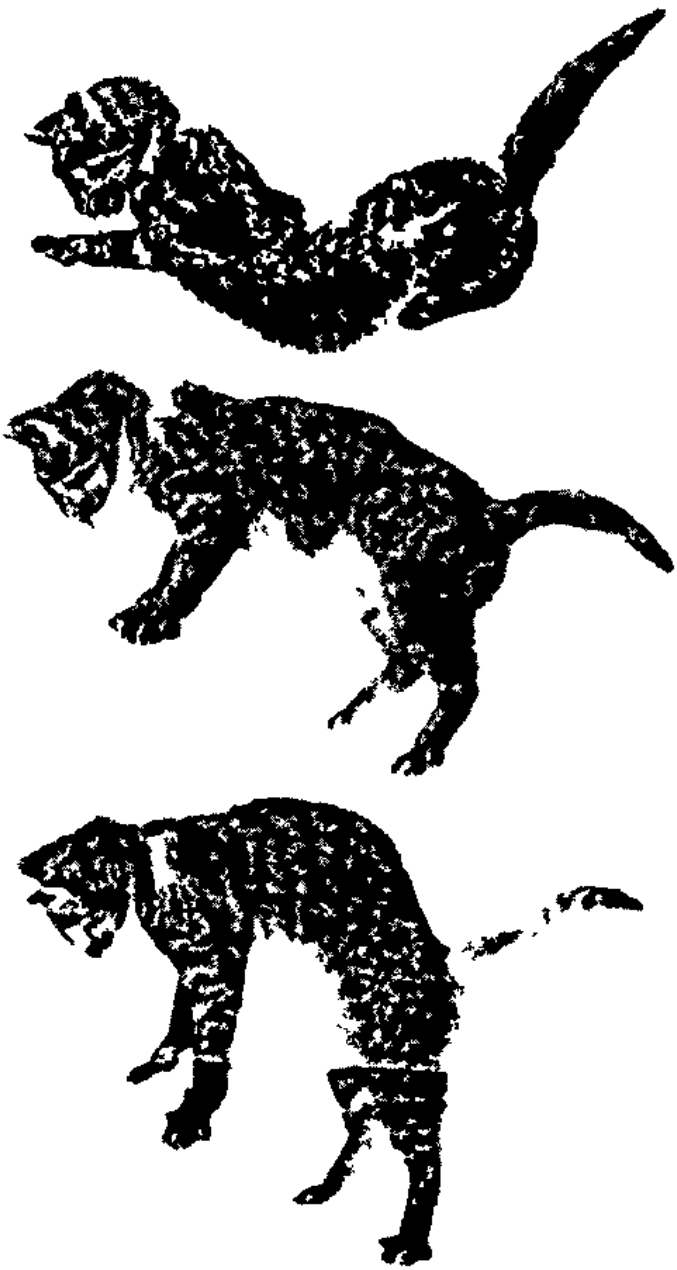

FIG 47 Righting reflexes in a normal cat dropped in the supine position from height of 1 2 m

ataxia, which at first is confined to hindlimbs but eventually also affects the forelimbs (figs. 47 and 48); and a progressive loss of rotational nystagmus, affecting at first the postrotational and later the perrotational

in a lot of streptomycin could not be noticed. Thus, of two streptomycin solutions that were equally potent in terms of streptomycin base, the one containing an added amount of streptidine definitely exerted greater neurotoxic effects in experimental animals.

The incidence of lot-to-lot variation in neurotoxicity depends to a marked degree on the purity of the sample (28). Though neurotoxicity is relatively frequent with material of a potency up to 400 μg of streptomycin base per milligram, it is virtually absent in streptomycin of a chemical purity exceeding 95 per cent, the neurotoxicity of such material being apparently due to that of the streptomycin molecule itself.

Use of streptomycin of the highest degree of purity seems therefore desirable. Although the pure drug will not provide better chemotherapeutic results than an equivalent dose of a material of lesser purity, it will

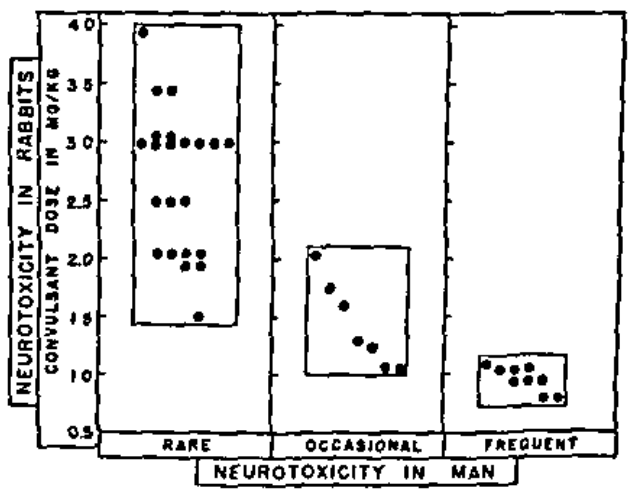

FIG 46. Relationship of neurotropic properties of streptomycin in animals and man.

minimize the variations in neurotoxicity. Any further reduction of the neurotoxic properties will require a change in the chemical structure of the streptomycin molecule itself, such as is the case with dihydrostreptomycin (29).

In animals, neurotoxic signs may be produced by repeated subcutaneous, intramuscular, or intravenous injection of 50 to 400 mg/kg of streptomycin base over a period of several weeks

The time required to develop a vestibular dysfunction varies with the size of the individual (daily) dose, the duration of administration (total dose), the sensitivity of the animal species as well as that of the individual animal, and the general conditions under which the test is performed (high temperature and humidity appear to shorten the time for the development of neurotoxic signs).

Of the many species examined (monkeys, dogs, cats, rabbits, guinea

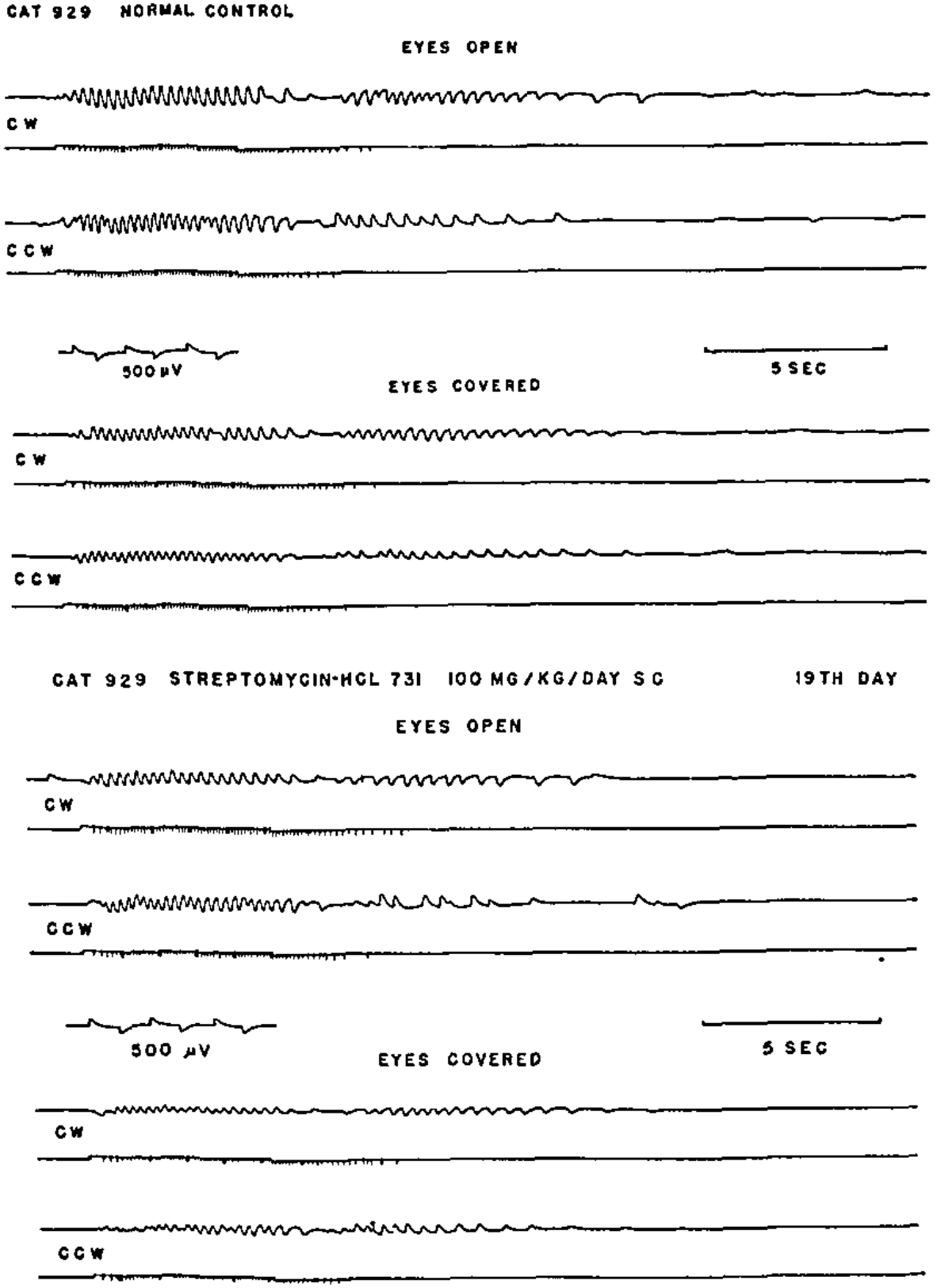

FIG 19 (a)
maximum speed
table (ca 1 rev
clockwise. (b) Nystagmus in same cat at onset of streptomycin intoxication. Recorded on day of appearance of ataxia. Minimal change in nystagmus, affecting post-rotational response (30).

response (fig. 49). The ataxia and other manifestations of vestibular dysfunction gradually disappear after the drug has been withdrawn; and

FIG. 48 Lack of righting reflexes in a cat severely intoxicated with streptomycin The animal was dropped in the supine position from a height of 1.2 m It had been treated for 18 days with crystalline streptomycin $CaCl_2$-complex in daily doses of 400 mg of streptomycin base per kg of body weight. The photograph was taken 50 days after the treatment was stopped.

cats which were periodically examined over 12 months after discontinuation of streptomycin administration showed a definite recovery of vestibular functions (fig. 50). In addition to the peripheral vestibular mechanism,

streptomycin also appears to affect certain closely related central, vestibular, cerebellar, and optomotor functions. Stevenson *et al.* (32) observed in dogs treated for 28 days with 170 mg/kg of streptomycin base a liquefaction necrosis of the ventral cochlear nuclei and the clumping of Nissl-like material in the majority of the neurones of these nuclei. Similar neuronal changes were found by these authors in tuberculous patients who had died after prolonged streptomycin therapy. The interpretation of the histological findings in these patients is complicated by the fact, however, that all of them manifested varying degrees of tuberculous involvement of the central nervous system.

The dose of streptomycin required to affect the auditory function of animals and man is much larger than that needed to produce changes in the vestibular mechanism. First affected is the perception of the high tonal frequencies this is followed by an impairment in the low-frequency range; and intermediate frequencies are affected last. Ability to perceive speech thus may be still normal at a time when reception of the other frequencies has been seriously disturbed. It is, therefore, highly important to use audiometer tests, if the earliest stages of the toxic effects of streptomycin upon the auditory function are not to be missed.

Although it is reasonable to assume that a specific neurotoxic effect is exerted by streptomycin on all animal species, it is more difficult to detect in some (guinea pigs, rats, mice) than in others (rabbits, cats, dogs, monkeys.) A latent disturbance of the vestibular mechanism, however, can be made manifest under certain experimental conditions which impose upon the animal the necessity to move in a manner to which it is not accustomed, such as swimming (33) or walking a tight rope (34).

The delayed neurotoxic effects just described can be produced by intravenous, intramuscular, and subcutaneous injection, but not by peroral administration, presumably because of the very small quantity of streptomycin absorbed from the gastro-intestinal tract.

If streptomycin is injected intracisternally or intrathecally in rabbits, certain neurotoxic effects develop which are distinctly different from those seen after repeated parenteral administration (4). The changes develop within 30 to 60 minutes. The animal is unable to remain in a normal erect position, becomes hyperexcitable, and reacts to touch with fast jerky movements. These signs are followed by muscular tremors, development of spontaneous nystagmus, and a sudden outbreak of epileptiform convulsions These seizures last 10 to 30 seconds and recur at frequent intervals of approximately 10 minutes. With larger doses (3 to 5 mg/kg) convulsions occur almost continuously and are often accompanied by loud screams. Animals that recover from the acute neurotoxic effects

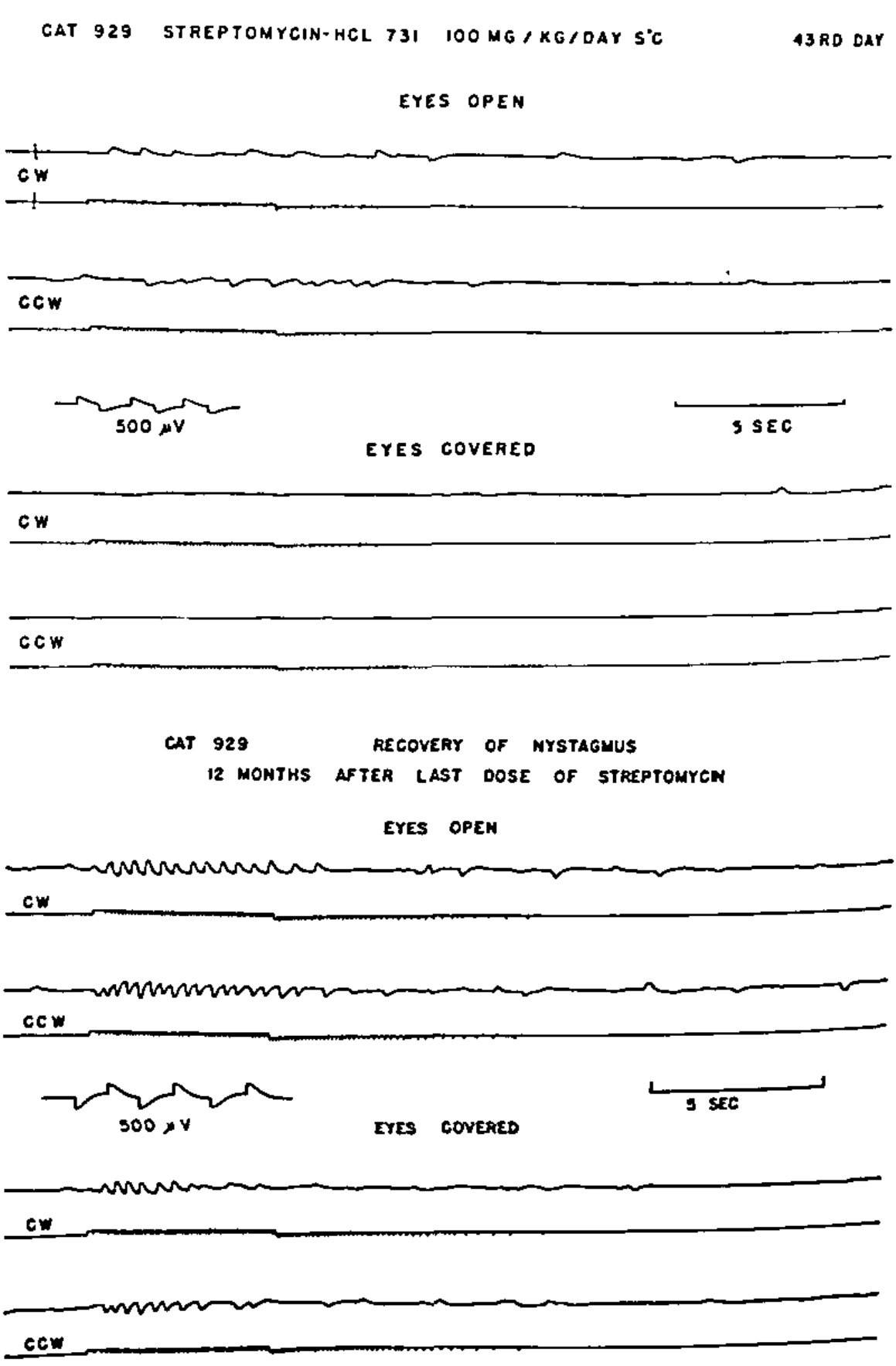

FIG. 50 (a) Electro-nystagmograms from same cat as figure 49 in severe streptomycin intoxication. Loss of vestibular nystagmus (eyes covered) virtually complete Optokinetic component still present in record with eyes open. (b) Partial recovery of nystagmus 12 months after last (50th) dose of streptomycin (31).

mycin in doses of 200 mg/kg, vestibular dysfunction developed only after 16 to 27 days (average 22 days). Similar results were obtained in dogs. Clinical findings (45, 46) corroborated the results obtained in animals and showed dihydrostreptomycin to be about half as neurotoxic as streptomycin (fig. 51), although possessing essentially the same antibiotic activity. In spite of the close chemical relationship to streptomycin and the fact that it is derived from the latter, dihydrostreptomycin is frequently well tolerated by patients who are allergic to streptomycin. Likewise, the eosinophilia often developing during streptomycin therapy appears to be less common with dihydrostreptomycin; an already established eosino-

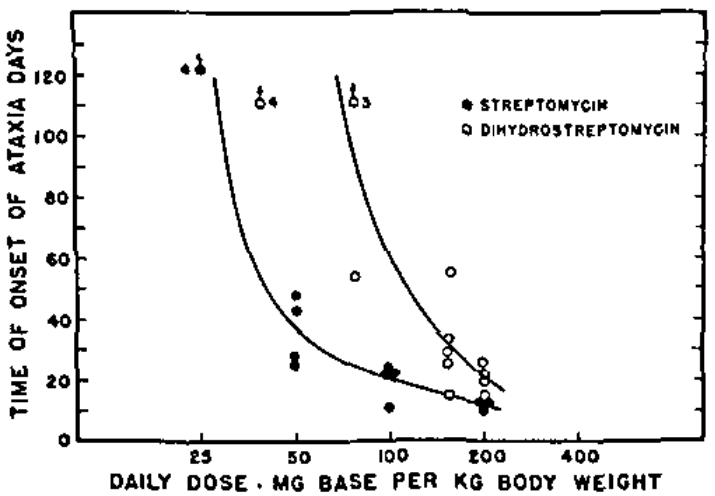

FIG. 51. Time of appearance of ataxia in cats treated with dihydrostreptomycin or with streptomycin as a function of the size of the dose employed Each point represents one animal, except where shown otherwise by numerals. Arrows indicate absence of neurotoxic effects.

philia decreased when dihydrostreptomycin was substituted for streptomycin.

Although some of the earlier and less pure lots of dihydrostreptomycin hydrochloride caused considerable pain and irritation at the site of injection, the presently available hydrochloride and sulfate are as well tolerated as streptomycin. Indeed, irritation tests in the rabbit's ear, using Kuna's technic (16), indicate that the sulfate of dihydrostreptomycin is considerably less irritating than streptomycin hydrochloride (47); preliminary clinical reports (48) seem to confirm these findings.

Streptomycin and dihydrostreptomycin do not appear to be interchangeable with regard to antigenic properties, but they cause the same response in the development of drug-fastness: bacteria which have become resistant to streptomycin are also resistant to dihydrostreptomycin.

remain normal and fail to show the long-lasting neurologic disturbances that are characteristic of the recovery phase from chronic streptomycin intoxication.

The convulsions following intracisternal injection of streptomycin resemble those observed by Walker *et al.* (3) after intracortical injection of penicillin and streptomycin. Similar convulsions may also be produced by intracisternal injection of guanidine, which, however, fails to produce the characteristic signs of delayed neurotoxicity (35).

Allergic reactions

Among the earliest side reactions observed in streptomycin-treated patients were elevations of body temperature, starting 1 to 2 days after the beginning of streptomycin therapy, eosinophilia, skin rashes, and development of an itching maculopapular eruption over the trunk and extremities (36, 37, 38, 14). Another frequent complaint, particularly following administration of the earlier and impure samples, was that of pain in the joints. None of these signs could be reproduced in animals. It would seem that they are of true allergic origin. Similar skin reactions have been frequently observed among nursing staffs and chemists who come in frequent contact with dry streptomycin (39, 40). As a rule, these dermatologic phenomena promptly respond to antihistaminic therapy, a further indication of their nonspecific, allergic origin.

Dihydrostreptomycin

The development of vestibular dysfunction and, less often, acoustic impairment after prolonged administration of streptomycin, has led to a search for streptomycin derivatives of equal antibiotic activity but lesser neurotoxic properties. The best compound prepared thus far is dihydrostreptomycin, made by catalytic hydrogenation of streptomycin. This new agent, first prepared by Peck, Hoffhine, and Folkers (41) and shortly afterwards by Bartz *et al.* (42) and Fried and Wintersteiner (43), has been investigated pharmacologically in animals by Edison *et al.* (29), Rake *et al.* (44), and Hobson *et al* (45); and in patients by Hinshaw *et al.* (46) and Hobson *et al.* (45). Although its chemotherapeutic, pharmacologic, and toxicologic effects closely resemble those of streptomycin, they differ from the latter with regard to the neurotoxic threshold dose. When the two drugs are given in doses containing equal weights of base, ataxia and loss of vestibular reflexes appear much later and are less severe with dihydrostreptomycin than with streptomycin. Thus, of four cats receiving streptomycin in doses of 200 mg/kg daily, all became ataxic within 12 to 14 days (average 13 days), whereas in four cats receiving dihydrostrepto-

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Section III

CLINICAL USES OF STREPTOMYCIN

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CHAPTER 16

CLINICAL INDICATIONS FOR STREPTOMYCIN THERAPY

Streptomycin has taken its place in the treatment of many infectious diseases that fail to respond favorably to any other chemotherapeutic agent or antibiotic. Two outstanding examples of such infections are tuberculosis and tularemia, but many others are listed below and discussed in this section.

DISEASES IN WHICH STREPTOMYCIN IS INDICATED

Abscesses due to mixed bacterial infections

Appendiceal abscess	Retroperitoneal abscess
Liver abscess	Subphrenic abscess
Perirectal abscess	Subhepatic abscess
Pelvic abscess	Tubo-ovarian abscess

Bacteremias

A. aerogenes	*Pr. vulgaris*
E. coli	*Ps. aeruginosa*
Kl. pneumoniae	*Salmonella*
H influenzae	Penicillin-resistant, streptomycin-sensitive, gram-positive bacteria

Bone and joint infections due to gram-negative bacilli

Brucellosis

Best results have been obtained in acute cases when used in combination with sulfadiazine

Endocarditis due to gram-negative organisms and penicillin-resistant, streptomycin-sensitive organisms

Infections of the gastro-intestinal and biliary tracts

Cholangitis	Diverticulitis
Acute cholecystitis	Ulcerative colitis
Pylephlebitis	

Genito-urinary tract infections other than pyelonephritis

Prostatitis	Seminovesiculitis
Epididymitis	

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Prostatitis	Seminovesiculitis
Epididymitis	

Granuloma venereum

Meningitis

H. influenzae
E. coli
Ps. aeruginosa
P. tularensis
Other gram-negative organisms

Ophthalmic infections

Conjunctivitis and corneal ulcer
Ps. aeruginosa
E. coli

Peritonitis due to single gram-negative organisms or mixed infections

Postabortal and puerperal sepsis

Prophylactic use prior to surgical treatment of gastro-intestinal tract lesions

Respiratory tract

Pneumonia due to gram-negative organisms
H. influenzae
H pertussis
Kl. pneumoniae
Empyema due to gram-negative bacilli
Lung abscess with mixed infection
Ozena and scleroma
Laryngotracheobronchitis
Infections of epiglottis

Salmonella infections

Infections of skin and subcutaneous tissues

Tularemia

Tuberculosis

Acute and chronic urinary tract infections

In selecting cases for treatment with streptomycin, it is essential that an etiologic diagnosis be made. Such a diagnosis provides a guide to the total daily dose of streptomycin and the duration of treatment. For example, in the treatment of tularemia, 0 5 gm a day for 5 to 7 days is usually adequate, whereas in the treatment of tuberculosis, 1 to 2 gm a day for 6 weeks is considered to be minimum treatment. In the treatment of other infections, different dosage schedules are desirable for optimal results, and they are discussed in connection with each disease and under the section dealing with dosage and methods of administration.

In mixed infections due to gram-positive as well as gram-negative organisms, it will be necessary in many instances to use a combination of both penicillin and streptomycin or streptomycin and sulfonamides. It is necessary, therefore, that as many of these cases as possible be studied from an etiologic point of view.

Once an etiologic diagnosis has been established, it may be well to deter-

mine the sensitivity of the organisms. This is not essential in every case, and treatment should not be postponed, in an acute infection, until this study has been made. It may become very important, however, in patients who have an infection with a susceptible organism (that is, gram-negative bacillus) that fails to respond to the average dosage schedule of streptomycin or when a patient has an infection due to an organism of the gram-positive group that is resistant to penicillin but sensitive to streptomycin in concentrations that are low enough so that streptomycin can be exhibited.

The clinical indications for streptomycin, then, are very broad, and all infections listed in the table and discussed in this section should be treated with streptomycin.

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Chapter 17
METHODS OF ADMINISTRATION AND DOSAGE

Local Application

The clinician may find it desirable on occasion to administer streptomycin in the form either of streptomycin hydrochloride or of streptomycin sulfate by means of local application. Though various concentrations of the material in aqueous solution have been employed, it would appear at the time of this writing that a satisfactory solution for local use is one which contains 2 gm of streptomycin per liter of distilled water (2 mg/ml). Such a preparation has been found useful for local application in the treatment of a variety of wounds infected by organisms susceptible to the action of streptomycin. In general, however, local antibiotic therapy, including streptomycin therapy, is limited in its application. First, the antibiotic, when applied locally, does not penetrate the deeper layers of tissue, and it is difficult, if not impossible, to eradicate infection when local therapy is relied on alone. When local therapy is used, the best results will be obtained when it is combined with the systemic administration of the antibiotic. Second, the clinician will do well to remember that the incidence of reaction, especially cutaneous reaction, is considerably higher after the local application of streptomycin than it is after the systemic administration of this antibiotic.

It should be emphasized further that solutions of streptomycin are to be used not for purposes of irrigation but rather for instillation. When solutions of streptomycin are used for purposes of irrigation, the material does not stay in contact with the infected surface for a sufficient time to permit bacteriostatic activity.

Intramuscular Administration

Intermittent, intramuscular administration is the method most commonly employed for the parenteral use of streptomycin. The most satisfactory stock solution of streptomycin for administration by the intramuscular route is one which contains 200 mg of streptomycin per milliliter of triple distilled water. Although some of the more highly purified prepa-

rations are well tolerated when administered subcutaneously, the intramuscular route is generally the method of choice. Some difference of opinion exists as to the frequency of the injections. In the beginning of our studies on streptomycin Nichols and the author (1) administered the material every 3 hours. In the treatment of most infections, excluding tuberculosis, it is now our practice, however, to administer the material every 4 to 6 hours. The investigations of Loewe and Alture-Werber (2) strongly suggest that 0.5 gm of streptomycin administered every 6 hours by the intramuscular route yields adequate streptomycin blood levels for the treatment of most infections. According to some investigators, satisfactory results may be obtained by administering the total daily dose by means of two injections. In the treatment of virtually all infections due to susceptible organisms, the recommended total daily dose is 1.8 to 2.4 gm of either streptomycin hydrochloride or streptomycin sulfate. It is seldom, if ever, necessary to administer streptomycin longer than 7 to 14 days. If the infection is going to respond favorably to streptomycin therapy, it will do so in this time.

What has just been said concerning the daily dose and the duration of treatment refers to the treatment of infections other than tuberculosis. Most authorities (3) on the use of streptomycin in the treatment of tuberculosis recommend an average daily dose of 1 gm. Furthermore, the duration of treatment of this infection often runs for several weeks (at least 4 weeks and at times as long as 8 weeks). In some instances the material has been administered daily for as long as 3 to 4 months. Furthermore, in the treatment of tuberculosis the entire daily dose is often administered in one or two injections.

On occasion it may be desirable to administer to the same patient a preparation containing both streptomycin and penicillin in the same solution. For such treatment the recommended stock solution contains 20,000 units of penicillin and 100 mg of streptomycin per milliliter. The solvent used in making these solutions is physiologic saline, rather than triple distilled water, which is usually employed when streptomycin is used alone. When this combined solution is used, the interval of the intramuscular injections is determined by the schedule of penicillin. Because penicillin is rapidly excreted, it is necessary to follow this schedule, which consists of making the intramuscular injections every 3 hours. At the same time, one will experience the cumulative effect of streptomycin. When one administers 2 ml of such a solution every 3 hours, the patient will be receiving 1.6 gm of streptomycin and 320,000 units of penicillin during a 24-hour period. Mixtures of streptomycin and penicillin are satisfactory when the stock solution is made up every 18 hours. The two antibiotics must not be allowed to stand in solution longer than 2 or 3 days,

because after that time there will be a loss of potency of penicillin, whereas stock solutions of streptomycin alone and stock solutions of penicillin alone, when kept in the icebox, are stable for at least 4 weeks. Stock solutions of either antibiotic older than 4 weeks should not be used.

Administration by Means of Hypospray (Jet Injection)

Hirsh *et al.* (4) have made preliminary reports on administration of streptomycin, as well as other substances, by means of hypospray. The principle is based on the fact that an extremely fine high-pressure jet is capable of piercing the human skin with only slight pain. The material to be injected is placed in a metal ampule, which has a capacity of 0.25 ml and is shaped like a blunt-nosed bullet. The tip of the ampule is held against the skin at the site of injection with the base locked securely in the apparatus. The plunger explodes against the rubber stopper, which forces the material out of the metal ampule and through the skin as a fine spray. The material is deposited subcutaneously and intramuscularly to depths varying from 0.2 to 2 cm, depending on the tension of the spring and the site of injection. In addition to the lack of trauma to the tissues, Hirsh *et al.* pointed out that the instrument eradicates the fear incident to injection by needle. Unfortunately, the maximal amount of streptomycin that can be dissolved in the volume contained in this apparatus is only 0.1 gm. The method, therefore, is not considered feasible in the treatment of infections requiring large doses of streptomycin. If the problem of dispensing larger amounts of the material can be surmounted, however, this may yet become an effective method of administering streptomycin, as well as other antibiotics.

Intravenous Administration

Streptomycin solutions have at times been administered by the intravenous route. Concentrated solutions thus administered, may at times produce considerable venous irritation, as well as systemic reactions. On the other hand, streptomycin may on occasion be given in a dilute solution by means of a continuous intravenous infusion. The total daily dose of 1.8 to 2.4 gm is administered in 2 liters of physiologic saline solution given at the rate of approximately 20 to 25 drops per minute. This method of administration is also often associated with considerable venous irritation and therefore is not considered the method of choice for the systemic administration of streptomycin.

Intrathecal Administration

Not all investigators are in complete accord concerning the value of the intrathecal administration of streptomycin in the treatment of meningitis

due to organisms susceptible to the action of this drug. It is the author's conviction that the objections raised to the intrathecal use of streptomycin in the treatment of meningitis are not based on fact. Certainly the slight irritation that may result is far outweighed by the improvement in clinical results when this method is employed. In other words, eminently better results can be expected when intrathecal therapy is combined with parenteral therapy than when parenteral therapy alone is used in the treatment of these patients. What has just been said applies not only to the treatment of coccal and bacillary meningitis, but also to meningitis due to *M. tuberculosis*. It was not until after intrathecal therapy was added to systemic therapy that satisfactory results were experienced in the treatment of the latter condition.

For intrathecal therapy it is recommended that adults receive 100 mg of streptomycin in 10 ml of physiologic saline solution. This amount should be administered every 48 hours until negative cultures have been obtained. For young children and infants the recommended dose of streptomycin for intrathecal use is 50 mg in 5 ml of physiologic saline solution. Roscoe and Gleeson-White (5) have recommended daily intrathecal therapy in the treatment of meningitis due to *H. influenzae*, and they further recommended that this procedure be continued until the cerebrospinal fluid has been sterile for 7 consecutive days. As already stated, the author and his colleagues have not found it necessary to administer the material more often than every 48 hours. Before the intrathecal injection is made, sufficient spinal fluid should be withdrawn to permit routine bacteriologic and other laboratory studies, including determination of the level of streptomycin in the cerebrospinal fluid. Furthermore, there is no contraindication to the intrathecal use of solutions containing both streptomycin and penicillin. When such a mixture is to be used, the 10 ml of saline solution should contain no more than 100 mg of streptomycin and 10,000 units of penicillin. It should be re-emphasized that the difficulties which have been encountered after intrathecal therapy have usually occurred when doses larger than 100 mg of streptomycin or in excess of 10,000 units of penicillin have been used for single intrathecal injections. Finally, it should be mentioned that at the time of this writing regular streptomycin is the preparation of choice for intrathecal therapy. Dihydrostreptomycin (6), although quite suitable and less toxic than streptomycin for systemic administration, is not recommended for intrathecal therapy.

Administration by Means of Nebulization

It is now clearly established that streptomycin, as well as other antibiotics, may be safely and satisfactorily administered into the tracheo-

bronchial tree by means of nebulization or by means of supraglottic instillation. Olsen (7) has reported considerable experience with streptomycin and combinations of streptomycin and penicillin in the preoperative preparation of patients with suppurative pulmonary disease and also has reported satisfactory results in the treatment of nonsurgical lesions of the lung, such as bronchiectasis. He emphasized, however, that one should remember that in the latter conditions one is treating the secondary infection rather than the primary disease. When streptomycin alone is used for purposes of nebulization, the preparation consists of streptomycin hydrochloride in a solution containing 200 mg of streptomycin per milliliter. When streptomycin is used in combination with penicillin, the preparation consists of 200 mg of streptomycin hydrochloride and 20,000

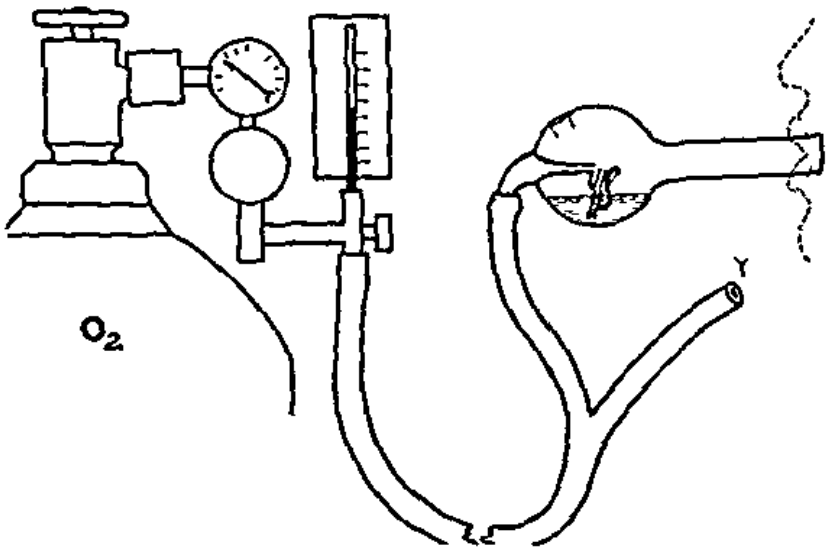

FIG 52
the source
is closed
"vaponefrin" type of nebulizer is depicted (7).

units of sodium penicillin per milliliter in the same solution. It should be emphasized at this point that the mixtures contain sodium penicillin and streptomycin hydrochloride. When streptomycin sulfate is employed, a cloudy precipitated solution, undesirable for this use, results.

The method of administration employed by Olsen is as follows:

A glass nebulizer was connected by means of rubber tubing to an oxygen tank equipped with a reducing valve and flow meter (fig 52). The solution was placed in the nebulizer, and the passage of 4 to 6 liters of oxygen through the nebulizer produced a fine mist The patient held the open end of the nebulizer in his mouth A Y tube was inserted into the tubing between the reducing valve and the nebulizer During inspiration the patient closed the Y tube The Y tube remained open during expiration, and oxygen escaped Thus, oxygen passed through the nebulizer only during the inspiratory phase of respiration, and loss of the drug was reduced to the minimum.

If the breath was held for a few seconds after each inhalation, a greater amount of therapeutic agent was retained in the bronchial tree The pressure necessary to nebulize solutions can be supplied by a small motor and air compressor as well as by the oxygen tank. In consideration of the results obtained in this series the possible therapeutic benefit of oxygen therapy should not be discounted entirely; however, for practical purposes any source of air pressure should be adequate. Air pressure has proved to be satisfactory for treatment by nebulization in the home.

Modifications of the standard glass nebulizer have been devised to prevent loss of too much of the drug during expiration. A special nebulizer with a large bulb may be used, or a collapsible rubber rebreathing bag may be attached to a standard nebulizer. Such modifications have been helpful for children and older persons who have found it difficult to close the Y tube during inspiration and to open it during expiration An automatic demand type of oxygen regulator also has been devised to deliver oxygen pressure only during inspiration.[4] The patients treated at the clinic whose general health has been good usually have preferred the standard nebulizer because it is easy to handle. For special cases an oronasal mask has been attached to the nebulizer.

Solutions of penicillin or solutions containing both streptomycin and penicillin can be nebulized at approximately 1 ml every 10 minutes. After short periods of rest the patient again nebulizes the material and many patients can nebulize 2 to 3 ml each hour. This is continued during that part of the day when the patient is awake. When the foregoing method has been used in the preparation of patients for pulmonary resection, the routine postoperative bronchoscopic examinations often have revealed that the bronchial tree was free of secretions. Likewise, good clinical results have been reported in the eradication of secondary infection in patients with bronchiectasis.

Reactions after nebulization of streptomycin are very uncommon. On occasion, patients may complain of a sore tongue after a few days of nebulization, and rises in temperature have been noted after instillations. No serious systemic reactions have been encountered, and it is well established that little or no streptomycin reaches the general circulation after its administration by means of nebulization. Olsen has pointed out that allergic patients would be more likely to experience reaction than nonallergic patients. In this connection, the report of Wiener and Lederman (8) of a case of local and systemic reaction in a patient after 9 days of streptomycin therapy by the aerosol method should be mentioned. Though this type of reaction undoubtedly will at times occur, it has not been a major problem. In fact, its occurrence after nebulization therapy is extremely rare.

Streptomycin may be instilled into the trachea in a manner similar to that employed for the introduction of iodized oil. Olsen recommended for supraglottic instillation 0.5 gm of streptomycin in 5 ml of isotonic solution of sodium chloride. When a mixture of streptomycin and penicil-

lin is desired, the amount used is 0.5 gm of streptomycin and 100,000 units of penicillin in physiologic saline solution.

Intrathoracic Administration

In the treatment of suppurative intrathoracic disease, such as empyema, it may be desirable at times to supplement systemic therapy with instillations of streptomycin into the pleural space. For intrathoracic treatment the daily amount of streptomycin usually recommended varies between 0.2 and 0.5 gm. The amount to be used should be dissolved in 40 to 50 ml of physiologic saline solution and instilled directly into the pleural space after thoracentesis. This procedure may be carried out once every 24 to 48 hours. When this procedure has been used in the treatment of empyema due to *M. tuberculosis*, rather sharp rises in temperature have been seen at times, and other untoward symptoms have been experienced (tuberculin-like reaction).

Intraperitoneal Administration

Under certain circumstances it may be desirable to employ antibiotic therapy by means of the intraperitoneal route. Generally, the prevention and treatment of suppurative disease in the peritoneal cavity lends itself well to the introduction of 5 to 10 gm of sulfathiazole at the time of abdominal laparotomy. On the other hand, there is no contraindication to the introduction of streptomycin alone or streptomycin in combination with penicillin directly into the peritoneal space. The recommended amount of streptomycin for introduction into the peritoneal cavity is 1 gm. This may be administered in a solution or, as recommended by Zingaro (9), 1 gm of streptomycin powder may be spread about in the peritoneal cavity. Zingaro expressed the opinion that the intraperitoneal use of streptomycin proved effective in the control of spread of peritonitis in seven patients treated in his service.

When one wishes to use streptomycin in combination with penicillin, the recommended dose is 1,000,000 units of penicillin and 1 gm of streptomycin. In connection with the intraperitoneal use of antibiotic agents, the investigator should remember, however, that these agents do not remain in the peritoneal cavity very long. They are readily absorbed into the general circulation. It is recommended, therefore, that in the treatment of peritonitis the intraperitoneal therapy be supplemented by systemic therapy, in which instance the antibiotic is administered by the intramuscular route.

Oral Administration

It is interesting that streptomycin is not absorbed through the intestinal tract. Even when large amounts are administered by the oral route, none

of the material reaches the general circulation. Furthermore, the drug is not destroyed by passage through the intestinal tract. The first property, therefore, renders the antibiotic quite safe for oral administration, and no evidence of toxicity will be experienced after its administration by this route. The second property ensures its activity against susceptible pathogens while it is present in the lumen of the gut. Reimann *et al.* (10), Pulaski and Amspacher (11), and Kane and Foley (12) have reported studies on the effect on the bacterial flora of the intestinal tract after oral administration of streptomycin. Sensitive microorganisms, as a rule, can be eliminated from the stool by the oral administration of streptomycin The stool will be free of these organisms as long as adequate doses of streptomycin are administered. The organisms, however, will promptly reappear when administration of the drug is discontinued. In general, there are two very definite indications, therefore, for the oral use of streptomycin. One is in the preoperative preparation of patients who are to undergo surgical procedures on the bowel. It is not necessary to administer streptomycin for more than 2 or 3 days before operation. The recommended dose is 0.5 to 1 gm every 6 hours by the oral route. The material may be satisfactorily administered in tomato juice or some similar liquid Administration can and should be continued for 5 to 7 days during the postoperative period.

Oral administration of streptomycin has also at times proved of considerable value in the treatment of certain complications which follow operative procedures on the bowel. For example, ascending infection of the urinary tract after ureteral transplantation to the bowel may at times quickly be brought under control by giving daily 4 gm of streptomycin by the oral route. In fact, the author has seen severe systemic reactions subside promptly after this procedure when the systemic use of streptomycin had not resulted in satisfactory control of such an infection.

Dihydrostreptomycin

Recently there has been considerable interest in dihydrostreptomycin (13–17). Not enough experience has accumulated at this writing to warrant any final statements concerning the value of this form of streptomycin. The chief interest in dihydrostreptomycin centers about the fact that the material appears to be considerably less toxic, especially to the eighth nerve, than regular streptomycin. It would appear that dihydrostreptomycin can be administered longer and in somewhat larger doses than streptomycin without producing eighth nerve toxicity. The investigator should remember, however, that if large doses are given and if the material is administered long enough, dihydrostreptomycin can produce this toxic effect. Furthermore, if the material is administered in the face

lin is desired, the amount used is 0.5 gm of streptomycin and 100,000 units of penicillin in physiologic saline solution.

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In the treatment of suppurative intrathoracic disease, such as empyema, it may be desirable at times to supplement systemic therapy with instillations of streptomycin into the pleural space. For intrathoracic treatment the daily amount of streptomycin usually recommended varies between 0.2 and 0.5 gm. The amount to be used should be dissolved in 40 to 50 ml of physiologic saline solution and instilled directly into the pleural space after thoracentesis. This procedure may be carried out once every 24 to 48 hours. When this procedure has been used in the treatment of empyema due to *M. tuberculosis*, rather sharp rises in temperature have been seen at times, and other untoward symptoms have been experienced (tuberculin-like reaction).

Intraperitoneal Administration

Under certain circumstances it may be desirable to employ antibiotic therapy by means of the intraperitoneal route. Generally, the prevention and treatment of suppurative disease in the peritoneal cavity lends itself well to the introduction of 5 to 10 gm of sulfathiazole at the time of abdominal laparotomy. On the other hand, there is no contraindication to the introduction of streptomycin alone or streptomycin in combination with penicillin directly into the peritoneal space. The recommended amount of streptomycin for introduction into the peritoneal cavity is 1 gm. This may be administered in a solution or, as recommended by Zingaro (9), 1 gm of streptomycin powder may be spread about in the peritoneal cavity. Zingaro expressed the opinion that the intraperitoneal use of streptomycin proved effective in the control of spread of peritonitis in seven patients treated in his service.

When one wishes to use streptomycin in combination with penicillin, the recommended dose is 1,000,000 units of penicillin and 1 gm of streptomycin. In connection with the intraperitoneal use of antibiotic agents, the investigator should remember, however, that these agents do not remain in the peritoneal cavity very long. They are readily absorbed into the general circulation It is recommended, therefore, that in the treatment of peritonitis the intraperitoneal therapy be supplemented by systemic therapy, in which instance the antibiotic is administered by the intramuscular route.

Oral Administration

It is interesting that streptomycin is not absorbed through the intestinal tract. Even when large amounts are administered by the oral route, none

of the material reaches the general circulation. Furthermore, the drug is not destroyed by passage through the intestinal tract. The first property, therefore, renders the antibiotic quite safe for oral administration, and no evidence of toxicity will be experienced after its administration by this route. The second property ensures its activity against susceptible pathogens while it is present in the lumen of the gut. Reimann *et al.* (10), Pulaski and Amspacher (11), and Kane and Foley (12) have reported studies on the effect on the bacterial flora of the intestinal tract after oral administration of streptomycin. Sensitive microorganisms, as a rule, can be eliminated from the stool by the oral administration of streptomycin. The stool will be free of these organisms as long as adequate doses of streptomycin are administered. The organisms, however, will promptly reappear when administration of the drug is discontinued. In general, there are two very definite indications, therefore, for the oral use of streptomycin. One is in the preoperative preparation of patients who are to undergo surgical procedures on the bowel. It is not necessary to administer streptomycin for more than 2 or 3 days before operation. The recommended dose is 0.5 to 1 gm every 6 hours by the oral route. The material may be satisfactorily administered in tomato juice or some similar liquid. Administration can and should be continued for 5 to 7 days during the postoperative period.

Oral administration of streptomycin has also at times proved of considerable value in the treatment of certain complications which follow operative procedures on the bowel. For example, ascending infection of the urinary tract after ureteral transplantation to the bowel may at times quickly be brought under control by giving daily 4 gm of streptomycin by the oral route. In fact, the author has seen severe systemic reactions subside promptly after this procedure when the systemic use of streptomycin had not resulted in satisfactory control of such an infection.

Dihydrostreptomycin

Recently there has been considerable interest in dihydrostreptomycin (13–17). Not enough experience has accumulated at this writing to warrant any final statements concerning the value of this form of streptomycin. The chief interest in dihydrostreptomycin centers about the fact that the material appears to be considerably less toxic, especially to the eighth nerve, than regular streptomycin. It would appear that dihydrostreptomycin can be administered longer and in somewhat larger doses than streptomycin without producing eighth nerve toxicity. The investigator should remember, however, that if large doses are given and if the material is administered long enough, dihydrostreptomycin can produce this toxic effect. Furthermore, if the material is administered in the face

of severe impairment of renal function, high blood levels will be obtained and neurotoxic effects will be experienced. In general, dihydrostreptomycin has about the same antibacterial activity as regular streptomycin. Organisms that are resistant to regular streptomycin are resistant to dihydrostreptomycin, and organisms can develop resistance to both preparations in about the same manner. The dosage and method of administration are approximately the same for both regular streptomycin and dihydrostreptomycin. The latter may be used locally, by the intramuscular route, by means of nebulization, and by the oral route. It should be reemphasized, however, as was pointed out earlier in this chapter, that the present available preparations of dihydrostreptomycin should not be administered by the intrathecal route. There is no contraindication, however, to the systemic administration of dihydrostreptomycin and the simultaneous use of regular streptomycin for intrathecal therapy.

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CHAPTER 18

STREPTOMYCIN IN THE TREATMENT OF TUBERCULOSIS IN MAN

This attempt to place between the covers of a book the record of a drug discovered less than 5 years ago is a daring and unprecedented one. It suggests a wealth of knowledge, established despite the haste of its gathering. It is particularly daring in the instance of tuberculosis, a disease so chronic in its habit that phthisiologists rarely do more than describe its victims as "apparently" cured, and so multiple in its manifestations that it required the discovery of the tubercle bacillus to unify its many forms beneath a single name. Because of these characteristics of tuberculosis, this chapter must be more tentative than many in this book, and its authors wish to make clear that it is being written during November 1948. That it can be written at all is due to two things: the development, during the years following 1938, of techniques (1) for the investigation of chemotherapeutic weapons in experimental animals, and the garnering of evidence on a—so to speak—mass production basis by clinical investigations in this country and abroad. One of these investigations has involved the cooperative efforts of sixty hospitals and many times that number of physicians.

The sequence of dates emphasizes the rapidity with which progress has occurred. The isolation of streptomycin and the demonstration of its effectiveness against cultures of *M. tuberculosis* were announced in January 1944, less than 5 years ago (2). Within a year, its effectiveness in the treatment of tuberculosis in laboratory animals had been demonstrated and reported (3, 4) and its use in the treatment of tuberculous patients had begun. Within the second year, reports of its effect on the first series of patients were published (5, 6). Scarcely 3 years have intervened since this latter date, during one of which supplies of streptomycin were most

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ment have been provided by 65 years of history. The effectiveness of streptomycin under these circumstances is now generally agreed on by investigators in this country (6, 11, 12, 13), in England (14), and on the continent (15). To take figures from a single series which combines the virtues of reasonable numbers and rather long observation (13): twenty-four of 100 patients with proved diagnoses were alive between 14 and 26 months after treatment had been instituted.

In the presence of meningitis, the immediate results of treatment may be dramatic. Patients who have been lethargic or comatose, and febrile, may become rational and afebrile within a matter of days. Not that such marked improvement is always, or even commonly, the case. As the English have pointed out in their report (14) of an admirable investigation being conducted under the auspices of their Medical Research Council, the patients with relatively mild symptomatology respond better to treatment than do those whose symptoms are advanced at the time treatment is initiated. A considerable majority of patients show some degree of initial improvement. Subsequent to this, they may continue to improve or they may relapse. These relapses may occur either at any time during the period of therapy or many months after it has been discontinued and an apparent "cure" has been achieved. It is, presumably, these late recurrences that account for the higher mortality rates (80 per cent) in the series which have been observed for relatively long periods (13) as compared with the lower mortality rates (40 per cent) of those series which have been observed for shorter periods (15). It seems probable that relapses and death are more frequently due to thrombosis of cerebral vessels, or to internal hydrocephalus caused by the organization of exudate at the base of the brain, or to reinfection of the meninges from cerebral foci to which streptomycin does not have access, than to a loss of sensitivity by the tubercle bacilli to streptomycin (12, 14). Indeed, there is some reason to think that the loss of sensitivity to streptomycin occurs less often in tubercle bacilli situated within the central nervous system than in those situated within the lung (14, 16), an observation which may be due to the apparent failure of streptomycin to penetrate to the site of focal intracerebral lesions from which the meningitis is derived (17).

The prolongation of life produced by streptomycin even in the patients who do not survive—a complete success in demonstrating the bacteriostatic effect of streptomycin although an incomplete one from the point of view of the patients—has resulted in introducing a new disease to medicine: chronic tuberculous meningitis, with a symptomatology and pathology quite its own. When clinical improvement occurs, it is paralleled by improvement of the pathologic changes in the cerebrospinal fluid; but this improvement is more laggard, for, although cultures commonly become

meager and restricted; and yet such extensive cooperative investigations have been conducted (7, 8, 9, 10) as to permit a single report (8) upon a series of nearly 3,000 patients treated with streptomycin for all the types of tuberculosis to which flesh is heir.

It will be observed that the steps in this study on streptomycin, rudely outlined above, have been logically and firmly taken: first, the laboratory demonstration of its effectiveness *in vitro*; second, the experimental proof of its usefulness in animals; third, the tentative clinical investigation; and fourth, as each of the preceding steps fulfilled its promise, the large-scale clinical studies which alone could produce adequate evidence in a disease of this nature. This is a satisfying sequence to the scientific mind but it is worth remark, since the point was once more proved in this instance, that it by no means assure[illegible]

[illegible] drugs, and at least a few in the field of tuberculosis, have been effective against disease in animals but impractical to use against the same disease in man. In the present instance the analogy from laboratory to animal to man happened to hold, but it was only in the clinic, the unavoidable final testing ground, that investigators learned of the true limitation and dangers which attend the use of streptomycin.

Although tuberculosis is identified by a common pathology and a common etiology, it represents such a dictionary of disease, as its symptomatology and course are modified by the tissue in which it exists, that it seems necessary to discuss each type separately. There is no particular or logical order in which these types need be considered, but it seems reasonable to select meningeal and miliary tuberculosis for first consideration, since it was here that the fundamental question at issue—the usefulness of streptomycin—has been most unequivocally demonstrated.

Meningeal and Acute Miliary Tuberculosis

These two types of tuberculosis have always involved, since their first recognition, a mortality rate so close to 100 per cent that their diagnosis has been regarded as suspect if the patient survived. Moreover, and this is particularly true of meningitis, the time between onset of disease and death is measured in weeks. These types therefore provided an ideal testing ground for streptomycin therapy, although a most severe one. The contrast between life and death is a criterion of effectiveness which must satisfy the most rigid statistician and, by this criterion, streptomycin has proved itself. Should but two or three of 100 patients survive, the ability of streptomycin to suppress or destroy the tubercle bacillus would have been demonstrated beyond reproach. The controls for this state-

ment have been provided by 65 years of history. The effectiveness of streptomycin under these circumstances is now generally agreed on by investigators in this country (6, 11, 12, 13), in England (14), and on the continent (15). To take figures from a single series which combines the virtues of reasonable numbers and rather long observation (13): twenty-four of 100 patients with proved diagnoses were alive between 14 and 26 months after treatment had been instituted.

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negative for tubercle bacilli within 2 to 3 weeks, abnormally high cell counts and protein concentrations continue to be observed. It is quite possible that these persisting observations are due in part to the irritant action of intrathecally injected streptomycin as well as to persistent infection. Residual neurologic stigmata, presumably as permanent as life itself, are also observed in some patients. They were a feature of the first case to be reported in the literature (18); they were present in three of the nine survivors in one series (13), in three of eleven in another (11), and in fifteen of thirty-three in still another (16); they are presumably a function of the destructive nature of tuberculous disease.

The outcome of acute miliary tuberculosis, when it is unaccompanied by meningeal disease and treated with streptomycin early in its course, is more generally favorable than is that of meningitis. The same clinical improvement occurs with the same promptness and, within 4 to 8 weeks, the pulmonary lesions, which may provide the only visible evidence of the disease, begin to disappear. If one starts with the assumption that streptomycin has some effectiveness, this clearing of the pulmonary roentgenogram is scarcely a subject for surprise, for it is precisely this sort of small, discrete, and new lesion which clears in experimentally infected guinea pigs and which one would anticipate being favorably affected in man. Although preexisting chronic focal lesions may be unaffected, the miliary seeding may become progressively less visible roentgenographically until it can no longer be seen. Pathologic confirmation of this healing has been demonstrated at necropsy (17), when small fibrotic scars may be found, although, elsewhere in the lung and in other organs, tubercles may be present and caseating. In one series [(13) and table 31], twelve of nineteen patients (63 per cent) proceeded to recovery and were alive 14 to 26 months after treatment was instituted. In another series of cases (16), thirty of thirty-eight patients (79 per cent) were alive after the completion of therapy. It should be stated at once (see below) that these very hopeful figures obtain only when meningitis does not accompany or follow the miliary disease.

When miliary tuberculosis is sufficiently widespread to include overt meningeal involvement at the time treatment is started, the prognosis is lamentable; twenty-three of twenty-five patients in one series [(13) and table 31] are dead, as are eighteen in another series of twenty-five (16) which have been observed for a briefer period. Even if the meninges are not obviously involved at the time treatment is begun, they may subsequently become so, and this development makes the prognosis much more serious. This sequence of events, miliary disease followed by a tardy meningitis which may develop during apparently effective streptomycin therapy or many months after its completion, had not been seen

prior to the use of streptomycin, for the very good reason that patients did not live long enough to develop it. The appearance of meningitis under these circumstances provides some supporting evidence for the belief that its pathogenesis involves an extension of infection from a focus of tuberculosis in the substance of the brain or spinal cord (19). It becomes of the utmost importance that the appearance of this complication be recognized at the earliest possible moment in order that treatment be started promptly; and, heretical as the suggestion would have appeared some years ago, it may well be sound practice to perform diagnostic spinal taps at regular intervals in the absence of meningeal signs and symptoms. It is by no means an infrequent complication, and materially affects the

TABLE 31

Results of treatment of acute miliary and meningeal tuberculosis

TYPE OF DISEASE	PATIENTS	LIVING*	DEAD			
		Total	After start of treatment			
			Total	0–1½ mo	1½–6 mo	>6 mo
Acute miliary	19	12	7	3	2	2†
Meningeal	43	9	34	16	11	7‡
Miliary and meningeal	25	2	23	14	6	3
Meningeal developing during or after treatment for miliary	13	1	12	3	4	5
Total	100	24	76	36	23	17

* All patients still living 14 to 26 months after start of treatment

† One patient in this group lost to observation but is presumed to have died.

‡ No tuberculosis of central nervous system in 2

favorable prognosis which was assigned to miliary tuberculosis in the preceding paragraph, of the two series mentioned there, one [(13) and table 31] originally contained thirteen additional cases in which meningitis subsequently developed and twelve of whom died to a man; the other contained thirty-one cases with twenty-six deaths.

We have been concerning ourselves largely with the prognosis of meningeal and miliary tuberculosis under streptomycin therapy. The situation can be roughly summarized in three sentences: 1. Approximately 50 per cent of patients developing meningitis have a life expectancy of at least 6 to 12 months, but these patients are not necessarily cured, and relapses occur with sufficient frequency that, after 2 years, the survivors are not likely to exceed one in five. 2. On the other hand, with the single and important proviso that meningitis does not develop, three in five or

even four in five patients who have miliary tuberculosis may be expected to recover. 3. Combined miliary and meningeal disease, developing either simultaneously or in sequence, still bears an ultimate prognosis little better than that of prestreptomycin days; the patients' lives may be—usually are—prolonged, but the chances of survival for 2 years would appear to be nearly negligible (13).

If one recalls that we are discussing a disease which has been constantly and rapidly fatal, these statements record a remarkable achievement, sufficient, certainly, to make the use of streptomycin not only mandatory, but mandatory at the earliest possible moment. Laboratory confirmation of the diagnosis should not be waited upon even if the cases become useless for further statistical purposes thereby. The results, however, are sufficiently short of perfection that one wonders if the weapon, clearly a sharp one, is being imperfectly used.

It has been customary, in this country, to employ the intrathecal as well as the intramuscular route for administration of streptomycin in the presence of meningitis. This was done on the same theoretical grounds that dictate the intrathecal use of penicillin, since appreciable concentrations of streptomycin do not pass through the normal choroid plexus into the cerebrospinal fluid. Tuberculous meningitis, too, has been seen to appear and progress in patients receiving maximal tolerated doses of streptomycin by the intramuscular route, and to recede when intrathecal treatment was added without the introduction of any other therapeutic factor. There is also the observation, made in the earliest series to be reported (11), that four of nine patients who received intrathecal medication survived, whereas the five who did not receive it died. This observation has been borne out in a controlled study upon 100 patients by English investigators (14) who found that 35 per cent of those who received combined therapy and but 11 per cent of those who received intramuscular therapy alone were in "good" condition. The intrathecal injection of streptomycin (0.1 gm) produces high peaks of concentration (750 to 2,000 μg/ml) in cerebrospinal fluid for several hours after the injection, which might seem to be desirable, and the drug diffuses readily into the cisternal and ventricular fluids (14). For these several reasons, combined therapy has seemed almost mandatory to observers in this country (7, 8, 9, 11, 12). The French investigators (15) appear to be divided in their opinion on this matter, some advocating it and others opposing it with equal feeling.

There are two points which might be made against combined therapy. In the first place, admittedly, in the presence of tuberculous meningitis, appreciable quantities of streptomycin pass the blood-fluid barrier and concentrations of 2 to 10 μg/ml may be found in cerebrospinal fluid (14, 16). It has been said (14, 16) that these concentrations increase as the

meningeal process becomes more acute and decrease as it subsides; and in application of this point it has even been advocated that an increase of streptomycin concentration in cerebrospinal fluid be used as an indication of relapse and for the resumption of intrathecal therapy. In the second place, admittedly, streptomycin is an irritant substance and its intrathecal injection can produce a considerable reaction. The degree of reaction appears to be correlated with the irritant properties of the solution, as indicated by the pain and induration which result from intramuscular injection, and to vary with the purity of the product. In the early days of one cooperative study (7, 8) when daily injections of 0.1 or 0.2 gm, or even more, were being made, the occurrence of root pain was common and at least four cases of temporary paraplegia were recorded. These incidents have been almost obviated by reducing the intrathecal dosage to 0.05 gm and the frequency of administration to alternate days, but it seems clear that even these dosages are partly responsible for the pleocytosis and increased protein concentration which cerebrospinal fluid displays during intrathecal therapy. In view of the evidence presented by the English investigators (14), it does not seem reasonable to hold intrathecally administered streptomycin responsible for the development of spinal block. Because of the desperate plight of patients with tuberculous meningitis, it would seem advisable to continue employing the intrathecal route until convincing evidence against its usefulness is adduced.

So far as the intramuscular dosage of streptomycin and the duration of its use in adults are concerned, it has been usual in this country to advocate a daily intramuscular dose of 2.0 to 3.0 gm for 120 to 180 days in both meningeal and miliary disease (7, 11, 12). Even larger doses have been used. The rationale of such intensive and long-continued therapy is the obvious one that the presence of a life-threatening disease outweighs any dangers attendant on toxicity. Insufficient evidence has been obtained by American investigators to demonstrate the superiority—or inferiority—of any one intramuscular regimen over another. The English investigators (14) used a variety of regimens, too many on too small a group to permit their drawing any conclusions in this regard; but they obtained some evidence, which they regard only as suggestive, that an interrupted course of therapy is superior to an uninterrupted course. Although the logic of such a course is not clear, it is interesting to note that the same idea is prevalent on the continent (20).

Various ideas have been expressed concerning the dose and duration of intrathecal therapy which should be used in the presence of meningitis in adults, but, again, there is insufficient evidence to permit a conclusion as to which is best. It is fairly well agreed that the individual injection should not exceed 0.05 gm and that three doses a week are adequate. Some

investigators (21) have recommended that intrathecal administration be continued throughout the intramuscular regimen, others (11) that it be stopped after 6 weeks; the French (15) seem inclined to stop after a series of six to eight injections or to omit it altogether, and the English (14) appear rather to favor interrupted courses.

It is, of course, altogether obscure why some patients with meningeal or miliary tuberculosis or both should recover and others, with an apparently similar infection and identical treatment, should not recover. Actually the physician treating such a patient can obtain only a vague conception of the pathologic conditions which are present. One can talk in terms of intensity of infection and native resistance to infection if one obtains any satisfaction from doing so. On the whole, it appears unlikely at this moment that the survival rate can be greatly increased by further juggling with the streptomycin regimen, and something new would seem to be clearly indicated. There are only two pieces of evidence to suggest what this might be: a demonstration that the simultaneous administration of one of the diaminodiphenysulfones (promin) and streptomycin is more effective in experimental tuberculosis in guinea pigs than is streptomycin alone (22); and the clinical demonstration—still incomplete—that another of the sulfones (promizole) and streptomycin produce impressive results in tuberculous meningitis in children (24).[1] The first of these leads has had some clinical investigation with results which are thus far conflicting. There is a favorable report in the Italian literature (23), but experience in this country has been discouraging (16): thirty-eight of sixty-one patients (62 per cent) with meningeal or miliary tuberculosis or both who received streptomycin and intravenously administered promin died within 12 months after the institution of treatment; thirteen of the twenty-three survivors had miliary tuberculosis without meningitis and might therefore have been expected to do well with streptomycin alone.

Many other leads deserve exploration: the use of interrupted regimens, the combination of streptomycin with other bacteriostatic drugs of the sulfone series and para-aminosalicylic acid, and the substitution for streptomycin of its less toxic reduction product, dihydrostreptomycin, so that larger doses can be safely administered Indeed, many of these possibilities are already under investigation But unless some one of them yields an unexpectedly dramatic result, it appears unlikely that any considerable further advance will soon be made in this country Large numbers of cases are required to weigh these several alternatives, and large numbers are not available here In France, on the other hand, 615 cases would

[1] As of December 1, 1948, eight of ten children with proved tuberculous meningitis are alive 10 to 20 months after the commencement of combined therapy with streptomycin and promizole

appear to have been accumulated in a single year (15). A well-organized and well-controlled study in that or some similarly unfortunate country would appear to be the best thing that could happen to the meningitis-miliary problem. In attempting to peer into the future it is sobering to recall that pneumococcal meningitis continues to exact a high mortality rate among its victims despite our possession of several nearly ideal antibacterial agents and effective specific serum therapy against the causative organism.

Pulmonary Tuberculosis

Tuberculosis assumes many forms, and pulmonary tuberculosis is the captain of them all—both in its incidence and in the number of lives it terminates. Although the mortality rate of tuberculosis has been reduced five-fold in this country since 1900, 52,000 deaths were ascribed to it in 1945, when streptomycin was first used, and 92 per cent of these were due to pulmonary disease (25). The situation is relatively much worse than this in many European and Asiatic countries, particularly since the recent war. In a country as small as Jugoslavia, for example, there are said to be as many deaths a year from tuberculosis as in the United States (26). These statements of emphasis are perhaps unnecessary for the reader; they are provided merely to indicate how important it seemed in 1945 and 1946 that an accurate evaluation of the effects of streptomycin be obtained as rapidly as possible. Too many remedies have already been introduced too carelessly and too uselessly into the therapy of tuberculosis. The prolongation of life in patients with meningeal and miliary tuberculosis was ample evidence that the drug had *some* effect on the tubercle bacillus *in vivo*. The next question that arose was the magnitude of this effect: would it cure tuberculosis in the sense of killing tubercle bacilli, or would it merely suppress their growth for a time, and, in either event, in what types of disease could this effect be produced? If the answer were sterilization, well and good, the millennium was at hand; but if it were bacteriostasis, the further, difficult question would arise of how streptomycin should be best integrated with other forms of therapy.

So far as pulmonary tuberculosis is concerned, because of the chronic nature of the disease, it will of course be a matter of years before the effectiveness of streptomycin can be evaluated by the final criterion of its effect on the mortality rate. But in the relatively short period since its introduction, there is already substantial agreement among investigators (7, 8, 9, 10, 11, 27) as to the immediate results which may be expected from its use, and the manner in which it should be employed.

This agreement emerges from four sources in the United States, two of them individual clinics (11, 27), two of them large scale cooperative studies

investigators (21) have recommended that intrathecal administration be continued throughout the intramuscular regimen, others (11) that it be stopped after 6 weeks; the French (15) seem inclined to stop after a series of six to eight injections or to omit it altogether, and the English (14) appear rather to favor interrupted courses.

It is, of course, altogether obscure why some patients with meningeal or miliary tuberculosis or both should recover and others, with an apparently similar infection and identical treatment, should not recover. Actually the physician treating such a patient can obtain only a vague conception of the pathologic conditions which are present. One can talk in terms of intensity of infection and native resistance to infection if one obtains any satisfaction from doing so. On the whole, it appears unlikely at this moment that the survival rate can be greatly increased by further juggling with the streptomycin regimen, and something new would seem to be clearly indicated. There are only two pieces of evidence to suggest what this might be: a demonstration that the simultaneous administration of one of the diaminodiphenysulfones (promin) and streptomycin is more effective in experimental tuberculosis in guinea pigs than is streptomycin alone (22), and the clinical demonstration—still incomplete—that another of the sulfones (promizole) and streptomycin produce impressive results in tuberculous meningitis in children (24).[1] The first of these leads has had some clinical investigation with results which are thus far conflicting. There is a favorable report in the Italian literature (23), but experience in this country has been discouraging (16): thirty-eight of sixty-one patients (62 per cent) with meningeal or miliary tuberculosis or both who received streptomycin and intravenously administered promin died within 12 months after the institution of treatment, thirteen of the twenty-three survivors had miliary tuberculosis without meningitis and might therefore have been expected to do well with streptomycin alone.

Many other leads deserve exploration: the use of interrupted regimens, the combination of streptomycin with other bacteriostatic drugs of the sulfone series and para-aminosalicylic acid, and the substitution for streptomycin of its less toxic reduction product, dihydrostreptomycin, so that larger doses can be safely administered. Indeed, many of these possibilities are already under investigation But unless some one of them yields an unexpectedly dramatic result, it appears unlikely that any considerable further advance will soon be made in this country Large numbers of cases are required to weigh these several alternatives, and large numbers are not available here In France, on the other hand, 615 cases would

[1] As of December 1, 1948, eight of ten children with proved tuberculous meningitis are alive 10 to 20 months after the commencement of combined therapy with streptomycin and promizole

streptomycin unless administration of the drug be combined with collapse therapy or surgical resection.

TABLE 32

Changes occurring in 1,009 patients having pulmonary tuberculosis treated with streptomycin by four different regimens

Regimens employed	1 2 0 gm 120 days	2 2.0 gm 60 days*	3 1 0 gm 120 days	4 0 5 gm 120 days
Patients	375	114	398	122
OBSERVATIONS BY INVESTIGATORS	INCIDENCE IN PER CENT			
Roentgenographic, before treatment:				
Progressive	65 4	77 4	76 8	78 7
Stationary	29 3	21 4	19 6	19.7
Regressive	5.3	1 2	3 6	1 7
Roentgenographic, at end of treatment:				
Markedly worse	0 8	1 3	3 3	1.6
Moderately worse	2 1	7 6	3 3	6.6
Slightly worse	2.9	5.1	1 8	4 9
	— 5 8	—14 0	— 8 4	—13 1
Unchanged	12.0	7 6	11 5	6.6
	—12 0	— 7 6	—11 5	— 6 6
Slightly improved	16 0	25 0	16 3	23 0
Moderately improved	30 4	22 8	28 3	33.6
Markedly improved	34 8	22.8	35 3	23 0
	—81.2	—76 6	—79 9	—79 6
Conflicting or uncertain	1 0	2 8	0 2	0 7
Roentgenographic relapses during treatment	6.9	0	9 5	17.2
Clinical changes during treatment				
Wt gain > 10 lb	42 0	—	44 9	41 1
Sputum decrease	70 0	61.4	74.1	57.1
Temperature to normal	57 9	52 2	63.6	51 4
Sputum conversion	40.6	44 6	35.1	—

* Changes recorded 120 days after starting streptomycin.

The roentgenographic changes summarized in table 32 (16, 31) then, are those which occurred in lesions of which the exudative components were the primary object of treatment. The table lists 1,009 patients treated by

involving seven (10) and sixty (8) hospitals. To the individual clinics belong the advantage of personal observation and the disadvantage of small numbers; to the cooperative studies belong the advantage of large numbers and the disadvantage of data collected by questionnaire from many physicians. In no one of these four studies were controls used, in the sense of alternate treated and untreated patients, and the question arises as to how much credence can be placed on their results in a disease so notoriously unpredictable as tuberculosis. Apropos of this point, two provisions of the protocols which governed the larger cooperative study (8), during the time when the value of streptomycin was still very much *sub judice*, should be mentioned: (a) all patients were to be observed for at least 60 days prior to therapy and were not acceptable for treatment if they showed radiologic improvement during that time; (b) the pretreatment regimen was to be continued unchanged and no new element—other than streptomycin—was to be introduced during therapy or for 4 months thereafter. Thus, it may be argued, each patient served as his own control in the sense that the course of his disease during and after treatment could be compared with its course before treatment; a reversal in this course—for example, from progressive or stationary to regressive—could therefore be properly attributed to streptomycin. It is, however, good to know that two studies which employ the treated-untreated control method have been undertaken recently (28, 29) and that their immediate results conform with those of the investigations under discussion; one (28) of the two has been published and confirms the belief that the less exacting studies of American investigators were interpreted correctly. Although the British study lacks the pretreatment observation period of its American counterpart (7), it is in many respects a model clinical investigation.

It is rather too much to expect that any chemotherapeutic agent can replace caseous material or fibrous tissue with alveoli. On that account and, even more, because experience with the first series of patients indicated it to be true (5, 6), investigators have eschewed the long-standing fibrocavernous type of disease and have selected for therapy those patients who, on the basis of roentgenographic observations, were believed to have a definite component of relatively recent, nondestructive, so-called exudative, disease. This decision has proved to be a wise one, the most favorable responses have been obtained in recent extension of disease, whether small or sufficiently large to be described as pneumonic, although, in this latter instance, if good results are to be anticipated, treatment must be begun before extensive caseation develops. Chronic disseminated nodular pulmonary tuberculosis has recently been described as responding extremely well to streptomycin (30). A sufficient number of cases with mixed disease have been observed to permit the general statement that the roentgeno-

patients in columns 1 and 2 of table 32 had been observed between 1 and 2 years after completion of therapy; the remainder had been observed less than 1 year. The patients in columns 3 and 4 have been observed a shorter time, half of them between 6 and 12 months, half of them less than 6 months after the completion of therapy. Sputum conversion, interpreted as negative cultures for at least 3 successive months without reconversion to positive, has occurred in only 35 to 45 per cent of cases, and an unknown number of these conversions may have been due to the introduction of collapse therapy in the posttreatment period. The incidence of relapses, occurring after the completion of therapy, has been 35 and 31 per cent, respectively, in the two series of patients (columns 1 and 2, table 32) who have been observed longest. Ninety-eight of the patients who relapsed have been retreated, with resulting improvement in forty. It seems probable that many of these relapses might have been prevented if collapse therapy or excision had been carried out at the ideal time.

The persistence of positive cultures in nearly half the cases and the occurrence of relapses in nearly a third makes it patent that, in spite of the rather constant roentgenographic and clinical improvement which is observed, streptomycin has a bacteriostatic effect on tubercle bacilli and does not destroy them. Indeed, this was implicit in the original experimental work, when a majority of the spleens in treated guinea pigs were found to contain viable bacilli (3, 4). It is, of course, equally the case with other modern antibacterial agents such as pencillin and sulfonamides.

Admittedly, there are occasional instances in which streptomycin—in conjunction with bed rest, without which it should rarely if ever be employed—may produce sputum conversion and complete roentgenographic clearing, and may therefore be regarded as definitive therapy for pulmonary tuberculosis. But in the vast majority of cases streptomycin must be regarded as adjuvant to some form of collapse therapy or more radical surgical procedure. The chief role which seems to be emerging for streptomycin is to make such surgical therapy possible sooner than would otherwise be the case, or to make it possible when it would otherwise have been impossible. The problem has become one of proper timing. Most authorities agree that when streptomycin is to be used, it is advisable to administer it until sufficient improvement has been obtained to permit whatever type of collapse or surgical excision appears to be appropriate at the moment, and to carry out such surgery as soon as it can safely be performed. This follows from the present belief that there is a time limit beyond which, usually, streptomycin can not be effectively employed. After about 30 days of streptomycin treatment, and continuing until about 120 days of treatment, an increasing percentage of cultures are isolated in which many of the bacilli are resistant to concentrations of streptomycin which, before

four different regimens. It will be observed that, whereas only 1 to 5 per cent were adjudged to be improving prior to therapy, 77 to 81 per cent improved during therapy and, contrariwise, whereas 65 to 79 per cent were becoming worse prior to therapy, only 6 to 14 per cent became so during therapy; here is the sharp reversal of trend on which this group of investigators based their conclusion that streptomycin can favorabely affect the course of pulmonary tuberculosis. When improvement occurs, it may be obvious on the 30-day roentgenogram but is rarely considerable before the roentgenogram taken 60 days after the beginning of treatment. Some of these observations of roentgenographic change by the observers have been subjected to a review by an unprejudiced jury of experts which confirmed their judgment. In table 32, the observed roentgenographic changes are graded in three categories: slight, moderate, and marked. It will be observed that the degree of improvement was more often recorded as slight or moderate than as marked and that marked improvement was not observed in more than 35 per cent of patients treated under any of the four regimens. The degree of improvement was startlingly similar in the series treated with 2.0 gm. and 1.0 gm. daily for 120 days (columns 1 and 3). It was somewhat less in those treated for shorter periods and with a lower dosage (columns 2 and 4), although the incidence of improvement was not affected by these changes.

The effect of treatment on cavities, except those associated with endobronchial disease, has been both various and unpredictable. Frequently, cavities which were recorded as closed or lost to view at one stage in therapy would reappear later in its course or after its completion. No constant, satisfactory result can be counted on unless concurrent or subsequent collapse therapy be added, and it is becoming increasingly clear that these destructive types of tuberculosis require collapse or excision more frequently than not.

Clinical improvement occurs more constantly and more promptly than does roentgenographic improvement, usually becoming obvious within the first 2 weeks of therapy. It may occur, temporarily at least, even in patients who fail to show roentgenographic improvement. Some of the more important changes are recorded in table 32; a decrease in cough and an increase in sense of well-being were encountered with such constancy that data on them are not recorded.

Two other matters should be commented on before it will be possible to assign, even in general terms, a role for streptomycin in the treatment of pulmonary tuberculosis: those concerned with the conversion of sputum or gastric contents from positive to negative, and those dealing with relapses. The validity of statistics on both points depends wholly upon the length of time over which the patients have been observed. Half of the

The closely intertwined subjects of dosage and toxicity are the final matters which require comment in this section. The latter of the two is treated in detail in another chapter, but it can scarcely be divorced from the present story. Just as the preliminary animal experiments were not designed to inform in the matter of resistance, so they could not be expected to inform of toxicity for man. The most important toxic manifestation was not even revealed by the clinical study which, during the spring and summer of 1946, so capably investigated the effects of streptomycin on acute nontuberculous disease (34), because the drug was given for such short periods. As soon as long-term treatment of tuberculosis was undertaken clinically, however, new and unique toxic results appeared (5, 6). An investigation into the toxic effects of a daily intramuscular dose of 3.0 gm continued for 120 days was initiated in the spring of 1946 (35), and the majority of investigators who began work after that time used, at the beginning, a daily dose of 2.0 gm. By the spring of 1947, as table 80 (7, 8) will indicate, it became clear that the vestibular apparatus was being damaged in a very high percentage of patients receiving this dosage. This complication had already been described (5, 36), but its severity and frequency had not been fully recognized. Accordingly, the very obvious expedients of exploring the effects of shorter periods of treatment, lower dosages, and less frequent injections were adopted. The results may be seen in table 75 (7, 8). They show a consistent decrease in important toxic manifestations with decreasing dosage, which, though not surprising, was highly gratifying. There then arose, as it always must, the question whether a corresponding decrease in therapeutic efficacy accompanied this decrease in dosage. In the opinion of one group of investigators (8, 31, 48) the therapeutic effects of 1.0 gm appeared indistinguishable from those of 2.0 gm in many clinical conditions and, although a second group (10) were inclined to think that some therapeutic advantages attached to the larger amount, there was complete agreement that the concomitant reduction in toxicity made the smaller dosage preferable. Further reduction of the daily dose to 0.5 gm resulted in a regimen which was, to all intents and purposes, free of toxicity and which certainly retained some therapeutic effectiveness, especially in the more responsive types of tuberculous disease. Whether any therapeutic efficacy was lost by this further reduction in dosage and, if so, whether the loss was compensated by the further reduction in toxicity, must remain *sub judice* at present. Alternate patients having pulmonary tuberculosis have been under treatment with 1.0 and 0.5 gm daily since April 1948 (16). One interesting conclusion which has emerged from the study is that a daily dose given in two injections is quite as effective as one divided into five injections; this is rather surprising in view of the current emphasis on constant blood levels but confirms experimental evidence which led to its investigation (37). Single daily injections are now

treatment, would easily have inhibited their growth. This phenomenon is described in detail in other chapters of this book but its complete elucidation may be doubted. Neither its pathogenesis nor its significance is yet clear. There are two quite different mechanisms which may underlie it: the development of resistant strains during treatment and as a result of it, or the multiplication of strains that are already resistant prior to treatment. If the former be true, shorter periods of therapy might avoid resistance; if the latter be true, some additional chemotherapeutic agent to which these strains are susceptible would appear to be necessary. Its significance is thought to be that it makes further streptomycin therapy useless [which is indeed the case with guinea pigs into which resistant cultures are injected (32, 52)] or, though of this the evidence is too frail, actually harmful (31); on the other hand, it has been demonstrated that a large quantity of sensitive tubercle bacilli do exist in patients who yield a resistant culture, and continued therapy might seem reasonable on that account. It is certain that many so-called resistant cultures are in actuality mixed populations.

This debate will take a long time to clarify itself and, meanwhile, considerable clinical evidence is appearing—no bit of it convincing by itself but impressive in its cumulation—that the appearance of resistant cultures does indeed mean that the patient will not respond to further streptomycin therapy (31, 27). Thus, to return to the point at issue, if one were to delay collapse procedures, waiting on maximal improvement, a relapse might develop and, the patient being now resistant to the effects of streptomycin, one would have lost the opportunity of using it to best advantage. It poses a very nice problem. With the evidence at hand, it would seem safer to regard streptomycin as a one-time weapon and to proceed with collapse therapy as soon as feasible and preferably before resistance develops. It is the same argument—the generally accepted thesis that it is folly to use one's single bullet against a jackal when a tiger is in the offing—which emphasizes the unwisdom of treating minimal tuberculosis with streptomycin or of using it in any situation that may reasonably be expected to respond to other forms of therapy, lest a life-threatening tuberculous episode subsequently develop. Meanwhile, every effort should be, and is being, made to overcome what has now become the head devil of streptomycin therapy. The search must be carried on rather in the dark until the mechanisms underlying resistance are understood, but much can be done in the dark. It now seems established that nothing can be accomplished by decreasing daily dosage (16, 31, 27), but studies on the effect of shorter regimens (for example, 42-day) and of the various permutations and

months or [for example, iodides (33)] are about to be undertaken

90 per cent of the 313 lesions are described as either healed or markedly improved. This presumably represents an accurate picture of what can be anticipated from the use of streptomycin in the "run of the mill" case;

TABLE 33

Changes occurring in 135 patients having tracheobronchial tuberculosis and 178 patients having laryngeal tuberculosis treated intramuscularly with streptomycin

Tracheobronchial			
Regimen	2.0 gm per day	1.0 gm per day	0.5 gm per day
Patients	67	59	9
BRONCHOSCOPIC OBSERVATIONS AT END OF TREATMENT	INCIDENCE IN PER CENT		
Healed (average 7 wk.)	46.3	72.9	67
Improved	41.8	18.6	11
Unchanged	10.4	5.0	11
Worse	1.5	3.5	11

Laryngeal			
Regimen	1.8-2.0 gm per day	1.0 gm per day	0.5 gm per day
Patients	61	90	27
LARYNGEAL OBSERVATIONS AT END OF TREATMENT	INCIDENCE IN PER CENT		
Healed (average 9 wk.)	37.7	55.0	48.0
Improved	45.9	36.1	44.0
Unchanged	8.2	2.8	4.0
Worse or unknown	8.2	6.1	4.0
CLINICAL OBSERVATIONS			
Hoarseness disappeared (average 5 wk.)	28.6	37.6	46.6
Hoarseness improved	44.6	47.7	46.6
Pain disappeared (average 2 wk.)	67.4	89.2	100.0
Pain improved	13.0	4.1	0.0
Dysphagia disappeared (average 2 wk.)	61.7	83.3	100.0
Dysphagia improved	15.0	7.4	0.0

the series is of considerable size, less care was taken to confine treatment to frankly ulcerative lesions than in the earlier series, and it includes about 10 per cent of noninflammatory, fibrostenotic lesions. Patients with actual ulcerations would appear to have responded best. It will be observed that

being used. This whole question of dosage will have to be reopened now that dihydrostreptomycin has become available and, if the substance proves as relatively free of vestibular toxicity[2] as would appear to be the case, the original irrational reason for decreasing dose would have disappeared; the old belief that 2 gm of any drug is, by definition, twice as good as 1 gm could be revived.

The most desirable duration of treatment is less a function of toxicity than of bacterial resistance to the drug. Originally, and quite arbitrarily, a period of 120 days was selected. If it could be shown that the incidence of resistance is markedly lessened by a decrease in duration of treatment, it would seem desirable to adopt a shorter period, even if there were some adverse effects on therapeutic efficacy or on the frequency of relapses. This effect on resistance has not yet been demonstrated. But a reduction in efficacy does not appear to accompany a reduction in duration of treatment to 60 days (8) and a further reduction to 42 days may diminish[3], but certainly does not destroy, the usefulness of streptomycin (16).

In general, the tendency over the last 2 years has been to reduce the daily dosage of streptomycin from 2.0 gm to 1 0 gm or even to 0.5 gm for reasons of toxicity, and to reduce the duration of treatment from 120 days to 60 days or even less for reasons of resistance. An optimal regimen has not yet been agreed on and is still the interesting object of pursuit, the possible variations having now been broadened by the appearance of dihydrostreptomycin.

Complications of Pulmonary Tuberculosis

Tracheobronchial, laryngeal, and alimentary

These several complications of pulmonary tuberculosis can be spoken of without equivocation. From the first reports (5, 6, 38, 39) until the present time (7, 8, 9, 15, 16, 27, 40) there has been uniform agreement as to the effectiveness of streptomycin on these lesions of mucous membranes.

All the lesions reported on in table 33 (8, 16) were diagnosed by repeated bronchoscopic or laryngoscopic observation and by the finding of positive cultures for acid-fast bacilli in sputum or gastric contents. They were treated for 120 days or until shortly after maximal improvement was observed to have occurred. Although the results are less uniformly successful than was true with the earlier series of fifteen cases (40), between 80 and

[2] Data presented before the Seventh Streptomycin Conference in April 1949 suggested that this freedom from auditory and vestibular toxicity is not as complete as had been supposed—even at a daily dosage level of 2 0 gm.

[3] Data presented before the Seventh Streptomycin Conference in April 1949 indicated that a daily dosage of 0 5 or 1 0 gm administered for 42 days was definitely less effective then the same dosage administered for 120 days.

losis and 105 cases of tuberculous enteritis have been reported from a single study (16, 41). In this study, lesions of the mouth and pharynx were occasionally diagnosed by biopsy, but more usually by the visualization of a typical ulcer in the presence of sputum or gastric cultures which were positive for tubercle bacilli. The diagnosis of enteritis was necessarily less definitive, and consisted of characteristic symptoms and roentgenologic findings in the presence of proved tuberculosis elsewhere in the body. The streptomycin regimen has been variable, with the same tendency to decrease daily intramuscular dosage (to 1 0 or 0.5 gm) and duration (to 42 days or less) as was remarked upon in connection with pulmonary tuberculosis; this was accomplished without obvious diminution of therapeutic efficacy. The oral use of streptomycin has been tried in a few cases of enteritis; in a sufficient number, certainly, to demonstrate that the effects are slower in making their appearance when the drug is administered by this route and, probably, that the results are less successful. A Swiss investigator (42) has suggested its use by enema. So far as results are concerned: four of each five oropharyngeal ulcerations healed completely; the symptoms of enteritis, often of such severity as to have required the use of opiates, subsided uniformly and often within a week; the intestinal roentgenograms showed some degree of improvement in at least two-thirds of the cases and a return to a normal-appearing mucosal pattern in some instances. With the disappearance of dysphagia and diarrhea there is a consequent increase in body weight among patients who have become emaciated (41) There is, be it said again, always the likelihood of relapse, although in the series under discussion it was reported at the surprisingly low figure of 3.6 per cent.

In these several complications of pulmonary tuberculosis, then, streptomycin accomplishes healing of the local lesions in a very high percentage of cases By virtue of this healing it provides symptomatic relief—relief of pain, of dysphagia, of diarrhea—in situations that are peculiarly difficult for both physician and patient. In the presence of the so-called tension cavities incident to endobronchial disease, its use may permit closure of these cavities. These are no mean accomplishments. But it should be emphasized that, by definition, one is treating complications. The relief of these complications may prolong life. The ultimate fate of the patient must depend on the fate of his pulmonary disease.

Tuberculous peritonitis

The relationship which has just been mentioned—that between the ultimate fate of the patient and the fate of his pulmonary disease—does not necessarily hold in the case of tuberculous peritonitis, for peritonitis may constitute the sole active lesion; its cure may then represent the patient's cure unless the caseous tuberculous focus to which it is usually secondary,

the results obtained with 1.0 gm a day appear superior to those obtained with 2.0 gm a day. It seems unlikely that this is the case. The difference may be ascribed to the presence of a larger proportion of ulcerative lesions, or to differences in the pretreatment duration of the lesion, which was shorter in the patients who received 1.0 gm a day (average 5.0 months) than in those receiving the larger dose (average 9.0 months).[4] Patients receiving 0.5 gm daily would appear to have done as well as those receiving larger doses, although the numbers in the former category are perhaps too small to warrant comparison. The patients reported in table 33 were treated by the intramuscular route alone. The aerosol route was abandoned early in the investigation when it was proved definitely inferior to the intramuscular route and when combined aerosol and parenteral therapy appeared to offer no advantages.

The symptomatic relief that followed the use of streptomycin in patients with laryngeal tuberculosis was one of the most gratifying aspects of the investigation, to clinician and patient alike. It occurred more promptly than did healing, when the latter was achieved, and with more constancy. Pain and dysphagia disappeared, in two-thirds or more of the patients, within an average of 2 weeks, although hoarseness, as might be expected on the basis of its pathology, disappeared more slowly and with less uniformity

In tracheobronchial and laryngeal tuberculosis, as indeed in all other forms of the disease that have been observed in sufficient numbers and for a sufficient period, relapses have occurred after treatment was discontinued The average incidence of relapse in 1,820 cases of the several types of tuberculosis which have been observed for approximately 12 months following the conclusion of treatment has been 18.8 per cent (31). One would have anticipated that the figure would be higher in the present instance, since the site of the lesions continues to be bathed in sputum containing tubercle bacilli in those cases (50 to 80 per cent) in which persisting conversion is not achieved This would not appear to have been the case.

As in the case of tracheobronchial and laryngeal lesions, so in the case of tuberculosis of the alimentary tract, the lesions of mucous membranes respond remarkably—it is difficult to avoid the word *dramatically*—to treatment with streptomycin Forty-nine cases of oropharyngeal tubercu-

[4] Such differences and others that cannot be detected in these data are unavoidable in studies that seek to compare any two groups of subjects which were selected at different times and often by different persons Many of the tabulations in this chapter and similar studies (10) have attempted to compare large doses of streptomycin administered to patients selected in 1946 and early in 1947 with smaller doses administered to patients selected later in 1947 or in 1948 Not only were criteria for selection of cases better defined in 1948, but collapse therapy was more freely used. Concurrent comparison of regimens has been in progress since the spring of 1947 (21).

Approximately four of every five sinuses and fistulae (78 per cent) healed within an average of eight weeks after streptomycin therapy was instituted and, almost without exception, the remainder were markedly or moderately improved. This is remarkable enough, but it is less so than were the results in the first fifteen patients of this series, in whom fifty-nine of a total of sixty sinuses healed (44). An attempt was made to trace this discrepancy in results by distinguishing, first, between sinuses and fistulae and, then, by dividing these into subgroups on the basis of their anatomic origin. The attempt was successful in the sense that it disclosed that sinuses heal more readily than fistulae (86 per cent as opposed to 57 per cent). It is the predominance of fistulae in the group treated with 1.0 gm daily which makes the results with this dosage (table 34) appear in-

TABLE 34

Chances occurring in 382 patients with 687 draining cutaneous sinuses and fistulae treated with streptomycin

Regimen	1.8–2.0 gm 120 days	1.0 gm 120 days	0.5 gm 120 days	0.2 gm 120 days
Patients	126	229	15	12
Sinuses and fistulae	257	403	15	12
OBSERVATIONS AT END OF TREATMENT	INCIDENCE IN PER CENT			
Healed (average 8 wk.)	87.3	69.6	60.0	83 3
Improved	7.8	23.5	40 0	16.7
Unchanged	4 6	6.9	0.0	0.0
Worse	0.3	0.0	0.0	0 0
Recurrence after healing	2.0	2 5	0 0	0 0

ferior to those obtained with the larger dosage; the appearance is deceptive. Sinuses originating in bone healed more frequently than those with other origins, and fistulae connecting with the pleural cavity did least well. The number of patients receiving a daily dosage of 0.5 to 0 2 gm is, relatively, very small, but the fact remains that ten of twelve sinuses treated with the smaller dose healed, although at a somewhat more leisurely rate. These same dosage schedules, administered for but 42 days, have not been in use sufficiently long to allow the evaluation of long-term results but, so far as immediate results are concerned, it would appear that there was very little choice between the shorter and the longer periods of treatment (16). Two-thirds of the patients reported in table 34 have now been followed for more than 6 months after the completion of treatment and one-quarter of them for more than 12 months; the relapse rate is only 4 per cent.

One important point which has emerged is the practicability, nay, the

is reactivated. A series of twenty-seven patients have been recently reported (8, 43) all but four of which were diagnosed as tuberculous by biopsy or by the culture of ascitic fluid. They received 1.0 or 2.0 gm of streptomycin intramuscularly daily, usually for 120 days. No correlation could be noted between the dosage or duration of treatment and therapeutic effectiveness. In twenty-two instances (81 per cent), the results were regarded as excellent, with subsidence of fever, when it had been present, complete disappearance of ascites, amelioration of abdominal symptoms, and striking gains of weight. Three of the remaining five patients were substantially unchanged and the other two became worse, one of them dying during a relapse that followed an initially favorable response. Posttreatment biopsies did not reveal evidence of persisting peritoneal tuberculosis in two of the three patients in whom they were performed.

Similar results were obtained in a somewhat larger series (16) of which this group of twenty-seven patients was a part.

Although the number of patients reported thus far is relatively small, for the good reason that tuberculous peritonitis is rather rare, there seems no reason to doubt that this series provides another example of the ability of streptomycin to heal superficial lesions of the membranes.

Draining Cutaneous Sinuses and Fistulae

The effectiveness of streptomycin on tuberculous lesions of mucous and serous membranes which has just been recited extends to lesions involving the skin and its underlying tissues, where its ability to produce healing is most remarkable despite the caseating destructive nature of the process. In prestreptomycin days, cutaneous sinuses often continued to drain for years. Their surgical treatment and closure were rarely possible. It is, now, a very rare sinus that will not dry and heal when treated with streptomycin, and this with extraordinarily small doses. The effect is so clear and at such complete variance with long experience that it is no more necessary to desire untreated controls in this instance than it is to speak of them in connection with meningeal and miliary tuberculosis. It was presaged in the first clinical report of streptomycin therapy (5, 6) in which all sinuses in three patients healed during treatment. A very much larger experience is now at hand, and its data are summarized in table 34.

Table 34 presents the results which have followed the use of streptomycin in 382 patients with 687 draining cutaneous sinuses and fistulae (8, 16). The tuberculous etiology had been established in each patient by biopsy of a tract or by culture of its drainage material. The average duration of the lesions prior to treatment was 45 weeks. All streptomycin was injected intramuscularly for 120 days and four regimens were explored, the smallest daily amount (0.2 gm) being used in an attempt to establish a minimal effective dose.

so marked as to produce evidence of destructive changes in the pyelograms, they are relatively unaffected by streptomycin, an analogy to its ineffectiveness in fibrotic and caseous pulmonary tuberculosis Lesions of the genital tract, especially prostatitis, are usually not improved by streptomycin, with the single exception that postoperative scrotal sinuses heal readily during its use.

TABLE 35

Changes occurring in 186 patients with genito-urinary tuberculosis during streptomycin therapy

Regimen	2 0 gm 120 days	1 0 gm 120 days	1 0 gm 42 days
Patients	86	54	46
OBSERVATIONS	INCIDENCE IN PER CENT		
Symptoms, at end of treatment			
Disappeared	32 5	13 0	21.4
Improved	51 5	72 9	65 6
Unchanged	11 3	8 7	10.9
Worse	4.7	5 4	2 1
Cystoscopic findings, at end of treatment			
Became normal	26.0	5 3	29 4
Improved	60 3	73 6	51 0
Unchanged	10 0	15 9	19 6
Worse	3 7	5 2	0 0
Pyelograms, at end of treatment			
Became normal	9 5	5 5	3 8
Improved	11 1	16 5	7 6
Unchanged	70 0	61 5	73 1
Worse	9 3	16 5	15 5
Bacteriologic findings at end of treatment			
Culture positive	15 3	19 6	45 0
Culture negative	84 7	80 4	55 0

One-third of the patients receiving the larger daily dose for 120 days have been observed for more than 12 months after the completion of therapy and 90 per cent for more than 6 months, there have been approximately 20 per cent bacteriologic and cystoscopic relapses in this group These relapses usually occur in patients having gross, established, renal disease At present, it would appear that patients having lesions of this sort which are clinically unilateral, and patients with genital tuberculosis, should continue to

desirability, of employing surgical procedures simultaneously with the start of streptomycin therapy or shortly after its inception. Pus should be evacuated and underlying necrotic tissue and bone should be removed whenever possible. This procedure was avoided during the early stages of the study so that the observed results might be those of streptomycin alone, but it was performed in one-third of the patients recorded in table 34.

There can be no doubt that draining cutaneous sinuses, and to a lesser extent fistulae, provide one of the most certain fields for the successful use of streptomycin; and the prevalence of these lesions, in certain parts of the world, makes it a very large field indeed. Their responsiveness to a daily dose of 0.5 or even 0.2 gm makes it possible to avoid significant toxicity. There remains here, as elsewhere with types of tuberculosis which are not necessarily a hazard to life, the physician's obligation to weigh the desirability of healing the lesion against the risk of causing streptomycin-resistant tubercle bacilli to develop.

Genito-urinary Tuberculosis

In this instance also, the results originally described (5, 6) in 1945 (five cases with four conversions) have been confirmed and greatly extended. A series of 186 cases has been recently reported (8, 16, 45) and is summarized in table 35 The diagnosis was established in all cases by positive culture or guinea pig inoculation, and the pretreatment regimen was unaltered other than by the addition of streptomycin. Both 2 0- and 1 0-gm dosages have been used for 120 days; the smaller dose has also been used for 42 days, but enough time has not passed since completion of this last regimen to allow its evaluation [5] There would appear to be insufficient difference in the effectiveness of these two dosages to compensate for the advantage gained by reduced toxicity with the smaller A daily dosage of 0.5 gm has not been employed.

Reasoning by analogy from the effects of streptomycin on other tuberculous lesions of the mucous membranes, one would anticipate that inflammation and ulcerations of the bladder wall would be favorably affected. This has proved to be the case Healing or marked improvement of cystitis has occurred in more than 80 per cent of the cases and, with this improvement, there has been a concomitant improvement in the most distressing symptoms (45) and in bladder capacity In the longer regimens, urine cultures have been converted to negative in 80 per cent of the cases, and this may prove to be true with the shorter regimen when observations have been sufficiently prolonged [5] On the other hand, when lesions of the kidneys are

[5] Data presented before the Seventh Streptomycin Conference in April 1949 indicate that the 12-day regimen is definitely inferior to the 120-day regimens in freeing the urine of tubercle bacilli

dosage or chronicity, the figures become too small to allow any clear decision as to which dosage is preferable or which stage of disease responds best. In view of the danger of encouraging the development of strains of bacilli which are resistant to streptomycin, it might seem wise to confine use of the drug to the more acute and progressive forms of the disease. Here, as elsewhere, relapses have occurred during the follow-up period; their incidence has varied between 10 and 25 per cent.

A Variety of Lesions

In this paragraph it is planned to dispose, in a sentence or so, of tuberculous *empyema* and tuberculosis of the *ear*, *eye*, *skin*, and *pericardium*. So far as empyema is concerned, it is reasonably clear that streptomycin, either alone or in combination with various methods of drainage, usually is ineffective therapy (6, 8), although it may permit definitive surgical treatment by decortication (16). As has been repeatedly suggested, this may well be due to the acid reaction of pus, for streptomycin is more effective in an alkaline than in an acid medium. On the other hand, streptomycin would appear to be useful in tuberculous otitis media, twenty-five of twenty-eight patients being reported (16) as apparently cured or improved following treatment. Tuberculosis of the eyes, skin, and pericardium deserves further investigation; the cases encountered in the cooperative study (16) are too few in number and the diagnoses frequently too suspect to warrant any conclusions. Several methods have been suggested for increasing the concentration of streptomycin in the aqueous and vitreous humors (46, 47). Favorable results have recently been described (50) in seven cases of lupus vulgaris following the use of calciferol and streptomycin. Three patients who had sarcoidosis have been treated; one of these has been reported (16) as improved, the other two as unchanged.

Comment

The introduction of streptomycin into clinical medicine has been peculiarly exciting to phthisiologists. Not only have they never had an "antibiotic" which was helpful in the treatment of tuberculosis in man, they have never had a proved effective drug of any sort. So far as chemotherapy is concerned, not only was their situation comparable to that of the syphilologist prior to the discovery of penicillin, it was worse than his position before the appearance of Ehrlich and arsenic. Phthisiologists have been dependent on rest in bed, which finds its climax in the "lung immobilizer" (51), and, more recently, on collapse therapy, which has become most definitive in the form of excisional surgery

Chemotherapy is a radical departure from tradition in tuberculosis, and on this account it might have been anticipated that a tuberculostatic

undergo surgical treatment if there is a reasonable chance of excising the lesion (45) and that the role of streptomycin is merely that of a prophylactic at the time of operation.[6] On the other hand, although observations must be carried on for much longer periods before definite statements can be made, the high percentage of persistent conversions indicates that streptomycin may be of permanent benefit in less advanced and early disease.

Orthopedic Tuberculosis

Two hundred and twenty-eight patients with tuberculosis of the bones and joints have been treated (8, 16) with the three regimens mentioned in the previous section. Despite this formidable number of cases, the series is less satisfactory as a basis for conclusions than are other series in this study. Improvement undoubtedly occurred in the local signs (inflammation, pain, swelling, limitation of motion) attendant on the infection; indeed, some degree of improvement in them was reported to have occurred in 90 per cent of patients during treatment, and the local signs were said to have disappeared or been markedly improved in approximately 50 per cent. There is some doubt about the roentgenographic changes, which are never easy to evaluate and always slow in making their appearance. It was the opinion of the investigators, an opinion confirmed by a jury review of roentgenograms in 100 cases, that disease which had been progressing prior to therapy tended to stabilize during the first 4 months after the beginning of treatment and to show improvement (usually slight) during the second 4 months The very gradual development of these changes, and the fact that ancillary surgical procedures were sometimes employed during and after treatment, make it difficult to attribute the results to streptomycin. This series deserves more careful analysis. It is perhaps fortunate that a control study—in the sense of alternate treated and untreated patients—in this field has been undertaken by the United States Public Health Service.

Lymphadenitis

In sixty-three cases of tuberculous lymphadenitis, proved by aspiration or biopsy, the patients have been treated with daily dosage of streptomycin of 0.5, 1 0, or 2.0 gm and have been observed for at least 120 days after the completion of treatment (8, 16). The nodes became nonpalpable in 21 per cent; in an additional 42 per cent they became markedly or moderately smaller during this period. When subdivided into groups on the basis of

[6] Other urologists (49) have advocated the use of streptomycin for a trial period of 60 days in the presence of a clinically unilateral renal lesion, even when the disease is

ance, [illegible]
if surgery became necessary.

reductions of dosage result in a striking reduction in the incidence of toxic manifestations, although these still appear in a small percentage of cases. Dihydrostreptomycin, which has but just become available, can probably be used at a daily dosage level of 2.0 gm, or even more, with a similar reduction of toxic manifestations.[3] But neither the decrease in dosage of streptomycin nor the introduction of its reduction product has had any effect on the development of resistance, which has now become the chief difficulty in maintaining the gains which streptomycin therapy can achieve.

The introduction of streptomycin has vastly simplified the treatment of certain types of tuberculosis; but these types are, for the most part, complications of tuberculosis, especially lesions of mucous and serous membranes, which are relatively rare. It has, on the other hand, complicated rather than simplified the treatment of pulmonary tuberculosis and of other forms of the disease in which surgical procedures play a role of steadily increasing importance. There is now the necessity of properly coordinating, in time, these surgical procedures with the administration of streptomycin. This is true because the tubercle bacilli become resistant to the effects of streptomycin during prolonged therapy

This resistance is the more serious because, once emerging, it is a persisting phenomenon. Certainly this is so in the laboratory, where on repeated subcultures and animal passage microorganisms retain the resistance of the parent culture and, in the dried and frozen state, retain it *at the original level for as long as 25 years. It would appear to be the* rule in man, although there are occasional records of a return of sensitivity over a period of many months Until some method is found for avoiding development of this resistance, its appearance must be borne constantly in mind in designing the best possible regimen of streptomycin for any particular case of tuberculosis. It is not difficult to make certain general statements. Thus, it is agreed that some 70 to 80 per cent of all patients receiving streptomycin for 120 days become resistant to the drug. And it is easy enough, and logical enough, to say that when surgical procedures are to be employed they should be employed before resistance has developed, whenever possible, so that the procedures may profit by the protective action of streptomycin, this is particularly true with such hazardous procedures as pulmonary resection Similarly, one may say that streptomycin should be avoided in the case of any benign tuberculous lesion which could be expected to respond to other forms of treatment, lest the patient, becoming resistant, be deprived of the benefits of streptomycin if some life-threatening tuberculous episode should subsequently develop. *But these generalities are very difficult to apply in any specific case* There is no way of foretelling at what date a given patient's bacilli will become resistant, or, indeed, whether they will become resistant at all A combination of both knowledge and luck is necessary.

drug would be slowly, perhaps inaccurately, evaluated. In the case of streptomycin, this would appear not to have occurred. Within 3 years after publication of the first clinical paper describing its use, it has been possible to write a chapter of this sort—tentative and incomplete in many respects, but unlikely to be regarded as complete nonsense within the next year or so. This relatively rapid evaluation of a new drug—rapid for tuberculosis if not for pneumococcic pneumonia—is partly the result of experience gained from the investigation of other drugs in other diseases. It is partly a result of the employment, here and in England, of cooperative methods of investigation which have yielded data, reasonably uniform rapidly, and in large amount; methods which were used, perhaps for the first time, in the beautifully organized study on penicillin and syphilis which was initiated during the recent war.

This is rather large talk. Moving from the general to the particular, let us see just what can be said. That streptomycin has a deleterious effect on the tubercle bacillus can be concluded from the prolongation of life, and the occasional survival, of patients with meningeal and miliary tuberculosis. That this effect is bacteriostatic rather than bactericidal can also be concluded, not only from the finding of viable bacilli in experimental animals after treatment, but also from the frequency with which relapses, due to bacilli still sensitive to streptomycin, occur in the clinic when treatment is withdrawn. This being the case—it has been said often, but cannot be said too often—streptomycin must be recognized as an adjunct to other forms of therapy rather than as, of itself, definitive therapy. What are these other forms of therapy? First, there is what, for lack of more precise terminology, is known as "*the defensive powers of the host*", *it is to this native* defense that one attributes the improvement which, initiated under streptomycin, continues after the drug is withdrawn. Second, there is bed rest, long the cornerstone in the treatment of active tuberculosis; if one had in hand a truly bactericidal agent it might be reasonable to disinter this stone, the therapeutic position of which is logical though not undisputed; having only streptomycin in hand, one would seem foolish to disregard bed rest and even to consider the treatment of tuberculosis with streptomycin on an outpatient basis. Third, and speaking now in terms of pulmonary disease, there are the various forms of collapse therapy (extensions, in reality, of the use of rest) and excisional surgery which also, of course, require institutional care.

The most obvious danger associated with the use of streptomycin, until, let us say, the fall of 1947, was the development of toxic manifestations, notably affecting the vestibular apparatus. That is no longer the case. It is generally, though not altogether, agreed that the daily dosage can be reduced from 2.0 gm to 1.0 gm, or even lower, without appreciable loss of therapeutic effectiveness in the more responsive types of disease. Such

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It is in this particular field—the avoidance of resistance—that the next advance in streptomycin therapy must come. The situation is intolerable, and the need urgent. The transference of streptomycin-resistant strains to previously uninfected persons is already a matter of record in two cases (16). It will in all likelihood become commonplace. It is serious in the sense that such individuals cannot be effectively treated with streptomycin, although in no other sense is their infection more dangerous. There is very good reason to anticipate some degree of success by the use of combined chemotherapy, particularly by the use of streptomycin and para-aminosalicylic acid. Numerous other paths in this field are already being explored. Some one of them may lead to the goal. Or some new drug may be found that possesses more virtue and fewer vices than streptomycin. It would be strange if the first antibiotic that proved to be bacteriostatic in the treatment of human tuberculosis were to be the best.

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scope, which is the cause of atelectasis, blocked cavities, and positive sputum. If patients suspected of having such lesions are given streptomycin,

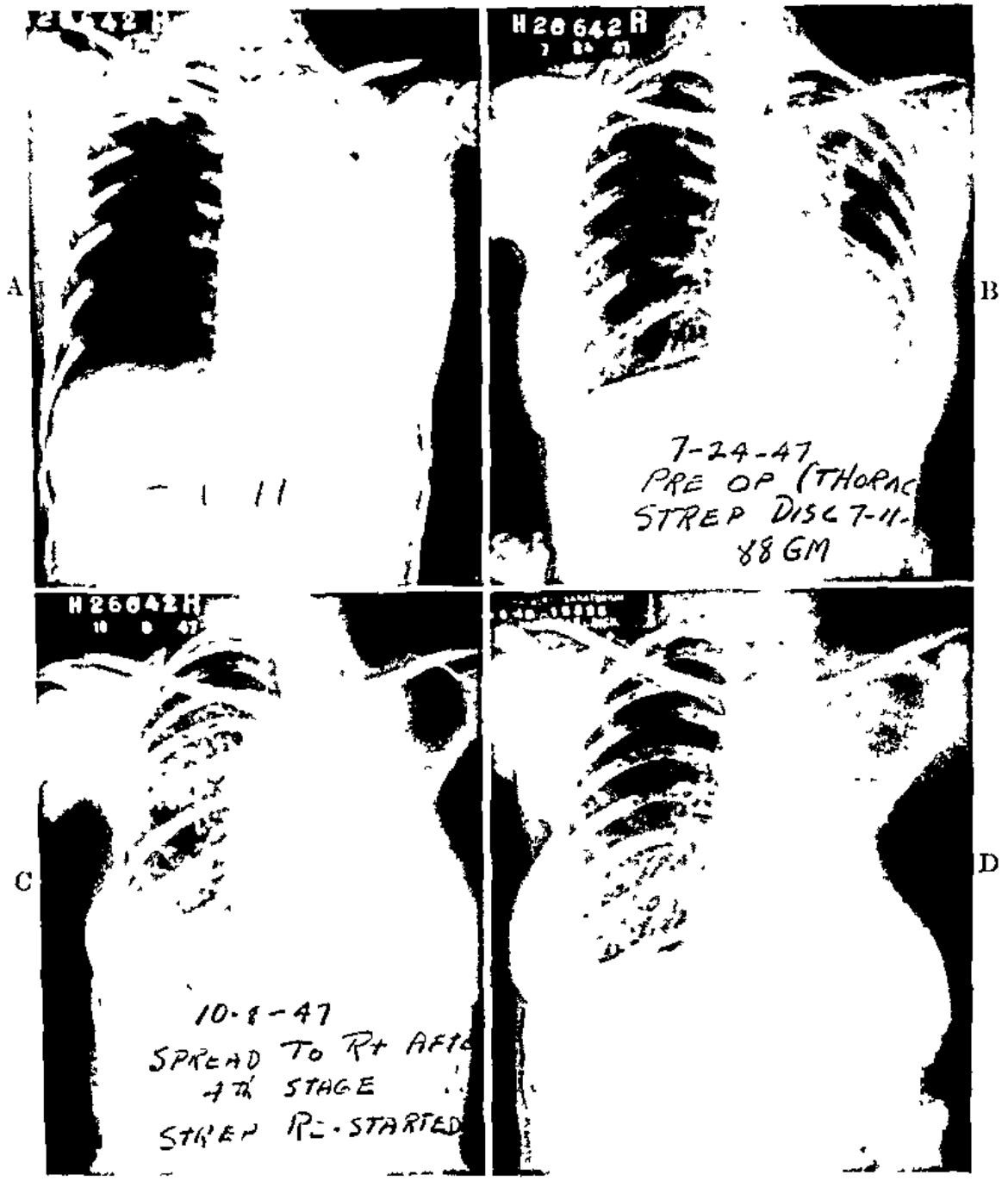

FIG. 53 (A), film before streptomycin; (B), reveals marked clearing two months after streptomycin—atelectasis gone, cavity remaining; (C), film showing spread following thoracoplasty in spite of previous streptomycin, (D), clearing after restarting streptomycin (Original)

with early and marked clearing of atelectasis and reduction in the size of cavities, it is certain that the diagnosis of endobronchial disease was correct and that following treatment the bronchi have again become patent (fig 53). If the results are negligible, however, it can be presumed in most

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CHAPTER 19

STREPTOMYCIN IN THE SURGICAL TREATMENT OF PULMONARY TUBERCULOSIS

Observations at the Wayne University Medical School and at affiliated hospitals of the Detroit Board of Health on more than 2,000 patients treated with streptomycin to date have provided a basis for some very definite general ideas about the proper role of this drug in the surgical treatment of pulmonary tuberculosis. An exact statistical tabulation of the patients is not yet possible. Streptomycin has been used extensively for so short a time that long-term results cannot be evaluated. Patients also are so widely scattered that accurate figures are not available.

The conclusions are based on the results of treatment given for various types and extent of disease.

Streptomycin was given to patients whose lesions, it was hoped, could be cleared without collapse or other surgical measures or in the hope that these procedures could be made less radical.

It was also used for patients in whom surgical procedures were contraindicated before treatment because of the extent of disease and the condition of the patient It was given here with the hope that improvement would be sufficient to allow institution of such procedures needed to complete recovery

Some patients also received streptomycin treatment to prevent spreads following surgical procedures or to clear such spreads after they occurred.

The treatment has been used extensively in patients with proved or suspected endobronchial disease These often heal as if by magic soon after streptomycin is started The number of bronchoscopies performed for these lesions has been markedly reduced Prolonged treatment with silver nitrate application is no longer necessary. Bronchoscopy is now done for diagnosis and again after streptomycin therapy. It is rare to find ulcers unhealed after treatment Some patients, however, have been found in whom the ulcer did not clear readily under administration of the drug. In these few instances, a sufficient amount of streptomycin probably was not given. This holds true for those few patients, also, who develop bronchial ulceration during and after treatment. There is frequently much endobronchial ulceration beyond the vision of the broncho-

lesions without cavitation has taken place after an arbitrary course of treatment, we have recently (unless contraindications are present) tended to continue with the streptomycin and have had some startlingly good results without adding collapse measures (fig. 55).

No one at present knows how much streptomycin a given patient needs or can tolerate before the lesion will clear or the organism becomes resistant. In this group of patients it is difficult to know definitely which course to follow. If use of streptomycin is continued, the patient may become resistant, and this may have been unnecessary. If collapse measures are not applied sufficiently soon, the disease may reactivate and spread because they were not used. If they are applied early without continuing streptomycin, there is the possibility they also may not have been necessary. We have tried to take the middle-of-the-road attitude, but we have, no doubt, added procedures which may not have been needed and have also withheld them too long with unfortunate results. As said before, it cannot be determined, after an arbitrary course of treatment that its continuance might not be essential, and *visa versa*. Reactivations have occurred following treatment which, we are sure, would not have taken place if more streptomycin had been given. In a number of instances, patients have been put back on streptomycin, and these lesions have cleared rapidly (fig. 53).

To prolong treatment one should, of course, know the sensitivity of the organism. But this takes time, and if sputum is negative, which is frequently the case, it is impossible. In many instances the organism has been found to be just as sensitive to streptomycin after the arbitrary course of treatment as before. In five patients who were resistant but whose sensitivity tests were not yet reported, streptomycin was restarted with excellent results (fig. 56). The fear of patients' becoming resistant to streptomycin, however, is always with us. In 750 patients treated with streptomycin at Maybury Sanatorium, 50 have become resistant. It is for this reason that streptomycin has been held in reserve when it seems possible that the disease may be arrested safely with simple collapse measures alone. Were this not the case, it is safe to say that the percentage of patients who have been spared collapse measures would be markedly increased. It is possible that, in the future, all patients will be treated with streptomycin when first seen, and without fear of resistance. Promin, amino salicylic acid, and dihydrostreptomycin help, but more refinements probably will be forthcoming It seems inconceivable, however, that anything will ever be able to permeate dense, fibrotic scar tissue surrounding tubercle bacilli. It becomes our duty, therefore, to see that patients are treated properly before this stage is reached. We believe that any soft

instances that the condition was either healed stenosis or fibrotic parenchymal disease or that insufficient streptomycin was given. As it is often difficult to differentiate between atelectasis and parenchymal disease by roentgenogram, some of the excellent results sometimes reported for old fibrotic lesions were probably in reality atelectasis from endobronchial disease. In tuberculoma, streptomycin has rarely been of benefit. All patients with fresh coin lesions should, therefore, have a resection, for in our experience about 50 per cent of these prove to be carcinoma, sarcoma, or cysts. About 40 per cent of those resected have been malignant.

A B

FIG. 54. (A), extensive unilateral tuberculosis. (B), appearance three months later after streptomycin—dose 0.5 gram daily (Original).

In patients with early, minimal and moderately advanced disease, streptomycin has decreased the necessity of simple collapse measures, such as pneumothorax, or phrenic operations, by more than 50 per cent (fig. 54). Even in more extensive disease, the results are sometimes so startling that further procedures are found unnecessary to complete the arrest of the disease, or much less radical measures are necessary. In the group of patients who have apparently been spared collapse measures, there are, of course, some who may need them later, whereas others who apparently need the assistance of some procedures to complete cavity closure and convert sputum, may become entirely well without them, for it is a routine observation that lesions continue to improve for months following discontinuation of streptomycin. If considerable but not sufficient clearing of

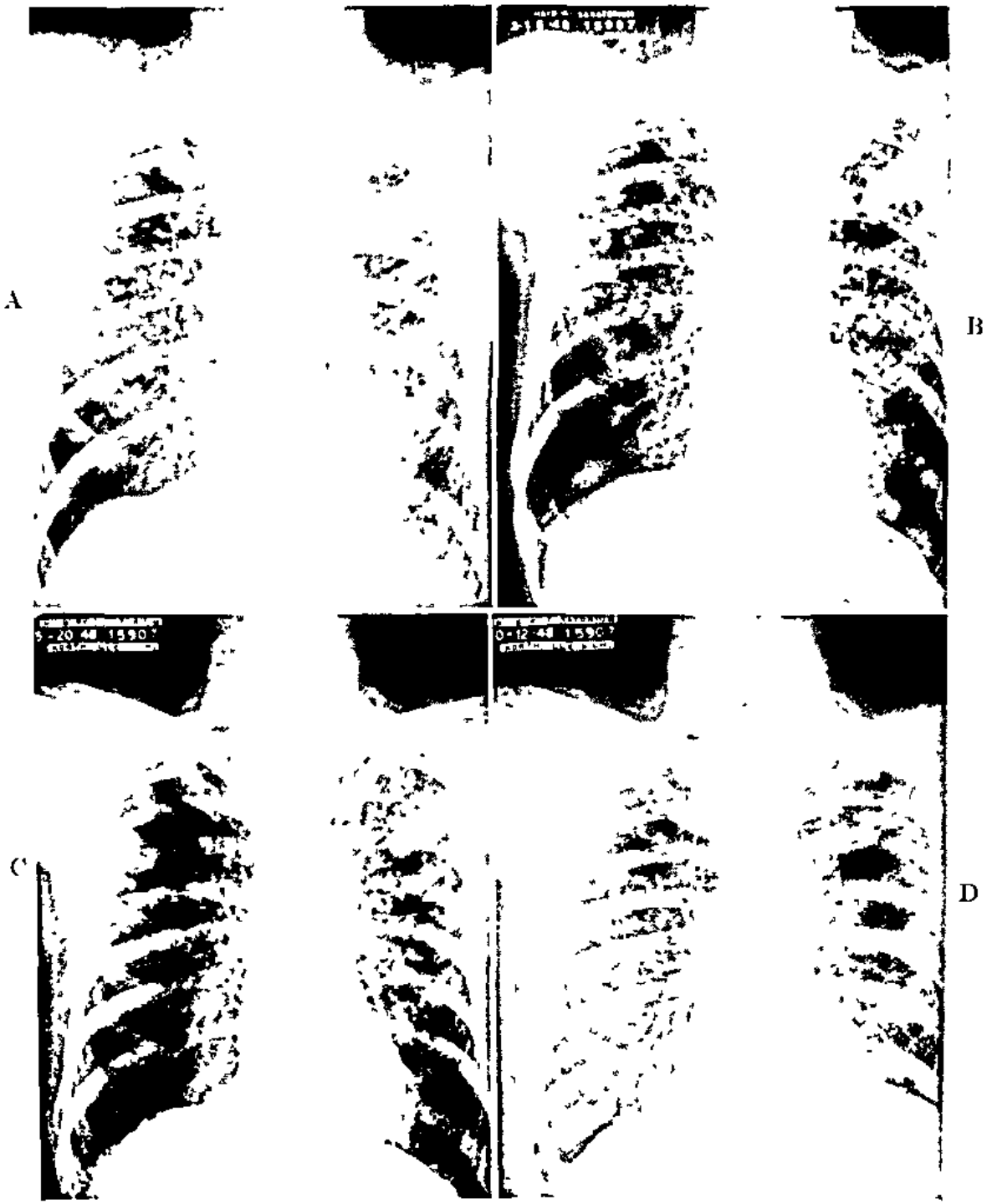


FIG 56 (A), extensive exudative bilateral tuberculosis; (B), clearing after one month of streptomycin, (C), marked clearing after a three months course of streptomycin Despite high resistance, streptomycin continued and a phrenic nerve crushing added to left (D), appearance after completion of second course of streptomycin, no cavity present—sputum negative (Original).

FIG. 55 Extensive bilateral disease in a very ill patient with positive sputum, temperature 101°F. (A), appearance before treatment, (B), one month after treatment with streptomycin; (C), four months after start of treatment (Original)

trary course of streptomycin with those who have not had such a course, because we are operating on much softer lesions often months earlier than we did before its use, and we are never sure the patient has had sufficient streptomycin before operation to prevent spreads. There can be no doubt that many months of hospitalization are saved by following this routine and that marked reduction in the financial burden to the family or community is achieved. Patients in whom a spread has occurred during or immediately following these operations, regardless of previous streptomycin, have cleared much more readily with administration of the drug, and with a marked decrease in the number of collapse measures necessary to assist clearing.

When so-called "spreads" occur, it is important, for statistical purposes, to distinguish between new, fresh lesions and reactivation of old lesions (or autotuberculinization), which are noted for their rapid clearing. A review of old roentgenograms is usually necessary to make this distinction. Of patients given streptomycin to prepare them for thoracoplasty, or to make this procedure possible, 20 per cent were found no longer in need of it. Of these, 5 per cent were classified as hopeless before administration of streptomycin. Of the extremely ill patients brought to thoracoplasty following streptomycin, 25 per cent were considered hopeless before administration of the drug. We have also found that with streptomycin there are fewer residual cavities following thoracoplasty. This is explained by the marked effectiveness of streptomycin in endo-bronchial disease, which causes blocked cavities that are often displaced but remain unchanged in size after one or two stages of thoracoplasty. These cavities frequently close after streptomycin. It should be used in such cases, however, before rebridged bone occurs to prevent approximation of cavity walls and healing. It must be kept in mind that the size of a cavity revealed by roentgenogram does not at all represent the amount of destruction of lung parenchyma. Cavities (especially blocked ones) have a tendency to balloon, and give a distorted idea of the amount of pathology present. Frequently, it is impossible to determine whether blocked or partly blocked cavities caused by active endobronchial disease exist. This is another reason why we believe streptomycin should be used as a prophylactic measure in all patients undergoing thoracoplasty. In spite of the excellent results obtained in endo-bronchial disease, we have found that, in extensive tuberculous bronchiectasis with invasion of bronchial walls and surrounding tissues, streptomycin was not very effectual.

Many patients received streptomycin prior to lung resection. A large number of these had extensive tracheobronchial disease or unstable lesions in the contralateral lung, which made resection impossible prior to its use. Most of these cleared sufficiently and were brought to resection with excel-

spreading lesions should be given streptomycin immediately, and even less active lesions which are not doing well under simple collapse measures should have it administered regardless of possible development of resistance. Roentgenograms and pathological specimens have indicated that lesions treated with streptomycin tend to heal by resolution or soft scar. If patients, therefore, are treated effectively early enough in the course of their disease, the problem of dense, fibrotic scar tissue should not present itself.

In spite of our stated middle-of-the-road attitude, we have tended lately, in patients with extensive soft disease with cavitation, for whom thoracoplasty or resection was considered, before streptomycin therapy, to be necessary ultimately, not to perform these operations if marked but not perfect clearing or closure of cavities occurs after streptomycin has been discontinued. We have tended, on the contrary, to add simpler procedures such as phrenic nerve crushing (fig. 56). Whether this is the proper course to follow can be determined only after much more time has elapsed and experience has broadened. In fact, what the future holds for all patients who have had streptomycin must await further observation.

We have found that the number of patients in whom major surgical procedures were possible was doubled by the administration of streptomycin. Many lesions, seen before administration of this antibiotic, were so extensive and of such character that collapse or resection measures could not be performed. Also, the condition of many patients was too grave, and there were complications such as gastro-intestinal, peritoneal, pelvic, genito-urinary, and bone disease as well as sinus tracts, contraindicating surgical procedures. Streptomycin is markedly effectual in such complications, and after these have subsided and the lesions have cleared sufficiently, surgical procedures have frequently been completed with good results. In many extremely ill patients, with high temperature and dyspnea (some almost moribund and in oxygen tents) the temperature rapidly became normal following administration of streptomycin. When the oxygen tents were removed, the patients continued to improve to such an extent that surgical procedures were possible later (fig. 56).

In a patient on whom major procedures are contemplated, streptomycin is used to clear or protect, during and after the surgery, soft lesions existing on the operation side or an unstable lesion present in the contralateral lung. We believe that in almost all patients undergoing major surgical procedures for pulmonary tuberculosis streptomycin should be used prophylactically. In negroes and others with known lack of resistance, the use of streptomycin under these conditions is imperative. Under this procedure, we have observed fewer spreads following major surgery. It is unfair, however, to compare spreads in patients who have had an arbi-

these patients had lung resections which necessitated cutting through the tuberculous ulceration in the bronchus. It has been proved that this is a very hazardous procedure, resulting in such complications as tuberculous empyema and opening of the bronchial stump. The remainder of the patients were given streptomycin as a prophylactic measure. Streptomycin was also used during the course of decortication of the lung because of extensive tuberculous pleural involvement and other factors (fig. 58) In these patients the postoperative course was smoother and pleural reactions were less marked and cleared more promptly than in those patients in whom streptomycin was not used.

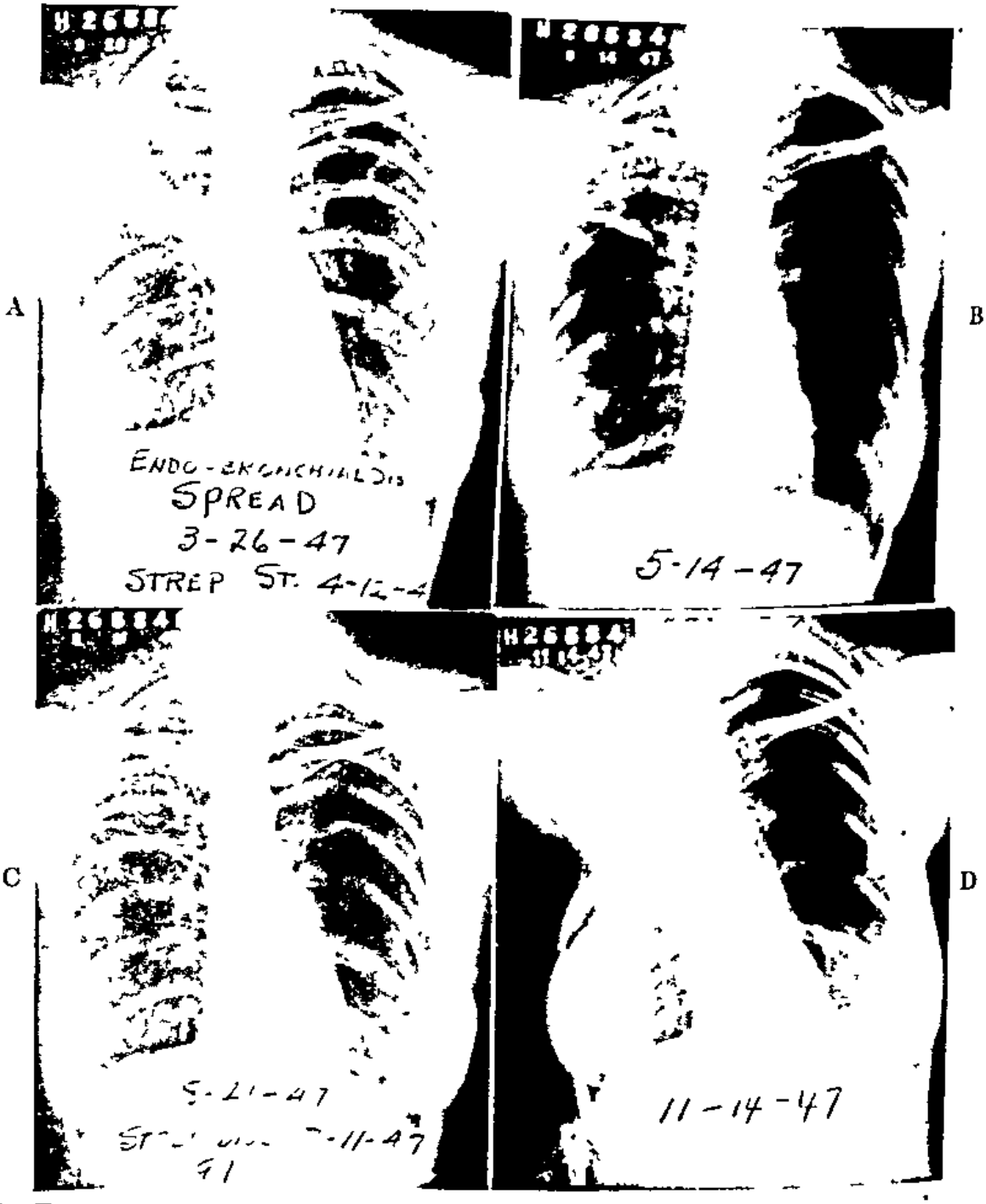
A B

FIG. 58. A), before decortication which was cura was found studded with tubere before and after decortication (B), appearance of chest following decortication (Original).

As has been mentioned, many patients who were thought, before streptomycin treatment, to need collapse measures of some sort did not need it afterward Others who were thought to need major procedures needed only minor ones after streptomycin therapy. Many others in whom collapse or surgery was impossible before use of streptomycin, were able to withstand application of such procedure. In some, however, there was no change in the pretreatment and posttreatment recommendations. In this group there was often considerable improvement in the lesions and symptoms, and the patients were made better candidates for the procedures recommended. In some, no successful collapse was possible before or after treatment. This group can be classified as failures (fig. 59).

As would be expected, our results were most striking in soft, mixed, and most recent lesions (figs. 55 and 56). Occasionally, however, in ap-

lent results. At operation there was no evidence of the previous bronchial ulceration in the severed bronchus in any case. In some patients with



Fi
culoi
(B) a
ance five months after admission Sputum still positive. Bronchus healed and lumen adequate for thoracoplasty (D), appearance after thoracoplasty (Original)

extensive tracheo-bronchial lesions where apparent stenosis existed, this has disappeared and a resection which was thought necessary was replaced by thoracoplasty (fig 57) Prior to the advent of streptomycin, many of

brought to surgery, and in many the outlook is hopeless. If streptomycin is given to this last group for symptomatic relief, the tendency will be to develop a group of patients who are resistant to the drug and to induce few permanent good results. Pressure from hopeful relatives often makes the decision difficult, however. In terminal patients it is probable that the temporary symptomatic improvement, the feeling of well-being, decreased cough, and lessened discomfort make its administration worthwhile. At least, the last days of such patients are made easier. Streptomycin does so many wonderful things when it is properly employed that its prestige will not be seriously damaged by following this philosophy. In so-called "good chronics," however, neither ill nor miserable, and with old fibrotic lesions, streptomycin should be withheld until surgical measures are contemplated. In this group there are, of course, many who do not need the drug, but small dosages given only during the operative period will seldom cause development of resistance. For the reasons given, we believe better results will be achieved in more patients if streptomycin is administered. This is especially true when there is any suspicion of perifocal pneumonitis or endobronchial disease.

In our experience, the results of streptomycin therapy, in properly selected patients, have frequently been so amazing that there always exists the fear that in the hands of the unscrupulous and those unfamiliar with its use and complications, it will be employed indiscriminately and cause more damage than can be offset by the results obtained.

One will not find perfect accord regarding the efficacy of streptomycin even among the best men in the profession. Those who are unfortunate enough to work in hospitals or sanatoria where most patients are of the old chronic, fibroid type, unfit for surgical measures, will not be enthusiastic about its use. It must be remembered, however, that all old chronics have become so, in most instances, because proper treatment was not instituted earlier. They were all, at one time, patients with soft, early lesions readily amenable to bed rest and collapse measures alone or with streptomycin.

One cannot conclude a discussion of streptomycin without dwelling on the complications which sometime follow its use. In the early days, excessive amounts were given, and there were many serious and permanent complications. Eighth nerve and vestibular involvement, kidney damage, and skin eruptions were common. This danger is still a reality. Too large dosages continued often for too long a time without proper tests of sensitivity are frequently employed. Much smaller doses, given once a day, or even 3 days a week, will accomplish the desired result, in most instances, with far less danger of complications. At the Herman Kiefer Hospital 0.5 gm is given daily for 12 weeks. At the Maybury Sanatorium 1 gm is given once a day, 3 days a week for 12 weeks. Possibly much smaller dosage will suffice in many instances. With the present dosage, a marked

parently old, fibrotic lesions, excellent results have been obtained. In almost all patients, some clearing of the lesions and clinical improvement

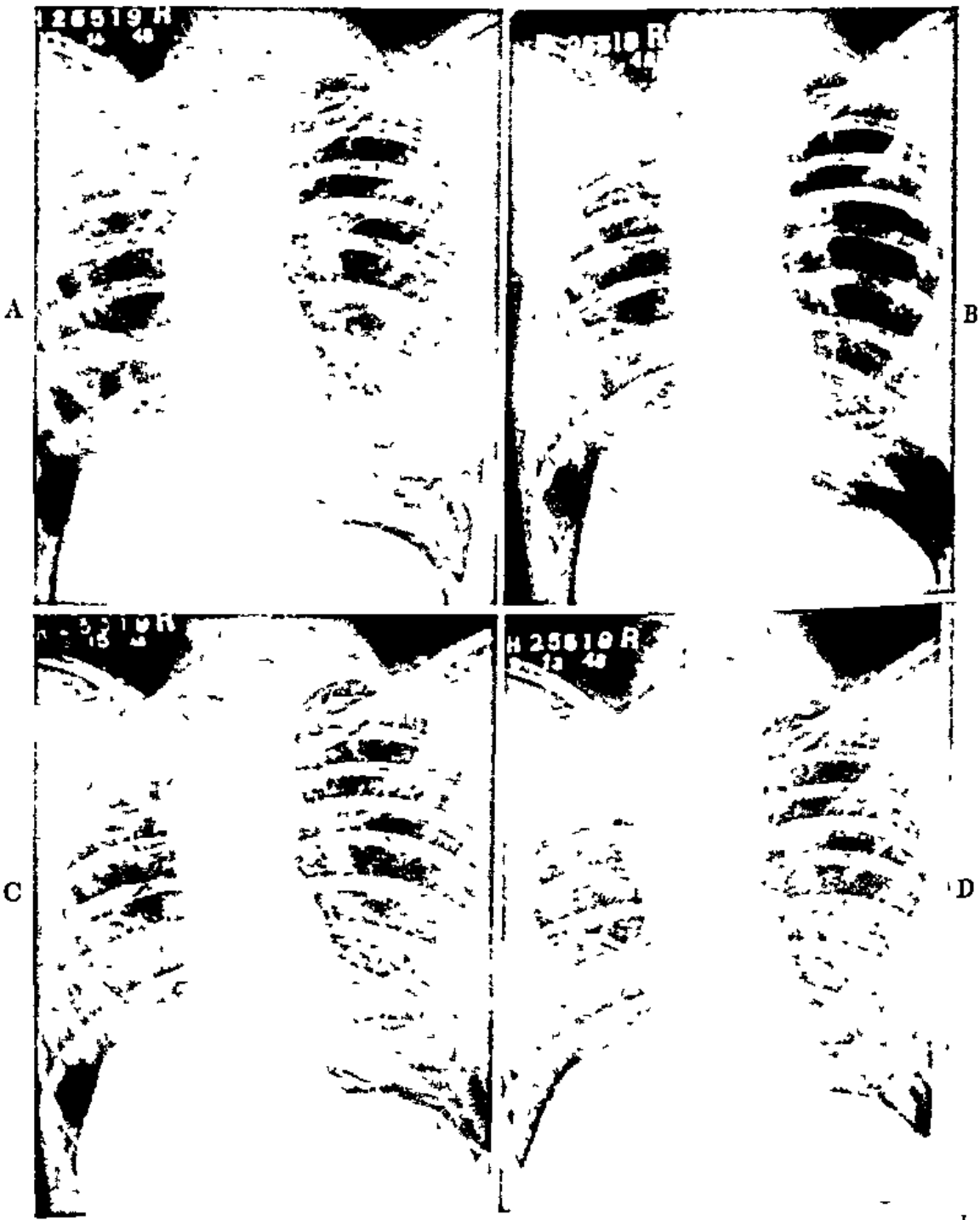


FIG. 59. (A), extensive bilateral tuberculosis marked by pneumoperitoneum and streptomycin (B), (C), (D), there has been no improvement from treatment (Original).

have been noted. One of our great problems is to know what to do with "old chronics" with bilateral disease not critically ill but often very miserable with excessive cough and other discomforts. Few of them can be

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CHAPTER 20

CLINICAL SIGNIFICANCE OF BACILLARY RESISTANCE IN THE TREATMENT OF PULMONARY TUBERCULOSIS WITH STREPTOMYCIN

Shortly after the tuberculostatic activity of streptomycin was demonstrated, it was found that tubercle bacilli could be made highly resistant to streptomycin by serial subculture in increasing concentration of the drug. It was learned also that resistant organisms were present in the secretions of patients who had received the antibiotic as therapy for tuberculosis. And it has now been established that resistant bacilli may be obtained from the great majority of patients who receive streptomycin daily during a period of 4 months.

It was anticipated early that the phenomenon of drug-fastness would be a serious limitation of the usefulness of streptomycin (1). Most investigators now assume that the laboratory demonstration of resistant bacilli reflects the presence of similar strains within lesions under therapy; and that these bacilli are also resistant to concentrations of streptomycin which are clinically feasible. Evidence, moreover, suggests that the organisms in an individual culture are of variable degrees of resistance, and that, in fact, many bacilli in the same culture are inhibited by very low concentrations of streptomycin. It is believed, further, that bacilli of dissimilar degrees of resistance are also present *in vivo*. It is reasonable to suppose that such varying proportions of sensitive and resistant organisms in a total bacillary population are intimately related to the clinical importance of the phenomenon of resistance. The value of further streptomycin therapy, when bacillary resistance occurs, and the effectiveness of retreatment with streptomycin during relapse should logically depend upon the relative preponderance of sensitive or resistant bacilli.

The techniques and limitations of laboratory methods useful in demonstrating the sensitivity of bacilli are fully considered in chapter 11. In this section an attempt will be made to elucidate the significance of the phenomenon of bacillary resistance by correlating the sensitivity of bacilli, as determined *in vitro*, with various clinical manifestions observed during

decrease in complications has been noted. Permanent significant damage virtually does not exist. Although temporary vestibular dysfunction and skin eruptions occasionally are encountered, they usually subside with the discontinuance of treatment. Treatment can usually be started again later without repetition of these manifestations. In some patients larger dosages may be required, but if these are used we believe they should be given on alternating days or weeks.

Patients with pulmonary tuberculosis when first seen present many varied problems. Some have early active minimal disease with almost no symptoms, others are moderately advanced, and some are far advanced, extremely ill and possibly dying. Numerous procedures are applicable to these conditions. Some need streptomycin, others do not. All of them, however, need bed rest, and, if sputum is positive, isolation is imperative Application of these procedures, however, along with the use of streptomycin is not uniform everywhere, and this is another reason for differences of opinion regarding the merits of its use.

In our experience the whole surgical approach to pulmonary tuberculosis has been revolutionized since the advent of streptomycin. Many patients are saved the necessity of collapse measures entirely, and others need much less drastic ones. We are now doing many more primary thoracoplasties and doing them earlier without preliminary phrenic operations and contralateral pneumos, which previously were necessary. Resections of segments, lobes, or lungs have been made much less hazardous procedures. This holds true also for decortication. Many patients previously denied any form of collapse or resection are now receiving these procedures. What is most important of all, however, is that the lives of many patients previously hopeless have been salvaged.

tubercle bacilli by an antimicrobial agent can be but a preliminary step, which must be followed by reparative processes dependent upon poorly understood attributes of the host. When streptomycin, therefore, is used for treatment of such lesions, the multiplication of bacilli is, indeed, suppressed, but the value of this bacteriostasis is limited by its relatively short duration. Viable bacilli remaining in such necrotic areas may resume growth, little may have been accomplished toward lasting healing, and pretreatment conditions may soon prevail. If the drug were bactericidal, necrotic lesions might eventually be sterilized; and though complete tissue repair never occurred, reactivation and relapse might be averted.

When, on the other hand, the recent bronchopneumonic lesion is treated with streptomycin, the result is usually excellent. In this situation, the favorable changes that ensue are due as much to the pathologic character of the lesion as to the inhibition of bacilli by streptomycin. Such bronchogenic metastases often contain relatively few bacilli, the exudate is largely reversible, and there is little or no destruction of tissue. The progression of such lesions to necrosis, when it occurs, is due, at least in part, to the rapid multiplication of bacilli. The arrest of bacillary growth by prompt administration of streptomycin tends to prevent necrosis and favors the resolution of the exudate while it is still largely reversible. It should be emphasized that a relatively brief period of bacillary suppression is sufficient for resolution of this lesion to manifest itself.

These facts have some bearing upon the therapeutic results that might be expected following temporary bacterial inhibition by streptomycin—temporary, either because therapy is interrupted or because bacilli have become predominantly resistant. It is not profitable, therefore, to consider the efficacy of streptomycin in the treatment of pulmonary tuberculosis except in terms of the pathologic characteristics of individual lesions. For evaluating therapeutic results, it appears best to classify pulmonary lesions according to the relative preponderance of two general components: the bronchopneumonic and the cavitary. This distinction is designed to correspond to the essentially prenecrotic and the necrotic lesion respectively. Admittedly, this is an oversimplification of the actual conditions in individual lesions, since bronchopneumonic areas may include necrotic foci of smaller or larger extent, and conversely, relative little necrosis may be present in cavitary lesions beyond the immediate "cavity wall." The division, however, is useful. Thus, the total pulmonary pathology of a patient may consist almost exclusively of a large cavity plus a small bronchogenic metastasis, the latter having been the immediate indication for streptomycin therapy. Or, the total lesion may be made up to widespread bilateral bronchogenic metastases which originated from a relatively small area of cavitation. When favorable changes in the *total* pulmonary disease

streptomycin therapy. Not all clinical varieties of tuberculosis lend themselves to this type of study. In the case of tuberculous sinuses, for example, the therapeutic results are so excellent that bacilli can be recovered only rarely during the course of streptomycin therapy. In the treatment of meningitis, on the other hand, the peculiar anatomical features of the lesion tend to obscure exact relationships between the sensitivity of bacilli and clinical changes. In the discussion that follows, data derived from treatment of pulmonary tuberculosis with streptomycin are utilized almost exclusively. The progress of this lesion can be followed with relative accuracy by serial roentgenograms; and bacilli are obtainable for study in most cases. Moreover, patients who receive streptomycin continuously for 4 months illustrate best the clinical significance of the sensitivity of organisms during therapy. In those who discharge organisms throughout the treatment period, the incidence of bacillary resistance to streptomycin is high, and the duration of therapy allows ample opportunity to observe the behavior of lesions. That group of patients, on the other hand, who receive shorter courses of streptomycin (30 or 42 days) is not particularly productive from this point of view.

Therapeutic Results and Bacillary Resistance

The value of a new therapeutic method for the treatment of pulmonary tuberculosis is difficult to assess, since the course of the disease is notoriously unpredictable in many instances. It was assumed from the beginning that the efficacy of streptomycin would best be demonstrated by administration of the drug to patients who had not improved by time-tried measures, more especially bed rest. The patients, therefore, who have contributed to our knowledge of streptomycin are a special group. One may infer that their native resistance to tuberculosis is lower than that of the average individual who acquires clinical tuberculosis, and that this relative immunologic deficiency may be reflected by the results of a type of therapy that is not definitive.

In addition, therapeutic results are intimately related to the pathologic characteristics of tuberculous lesions with their individual inherent tendency either to heal or to remain relatively indolent. In contrast to pneumococcus pneumonia, the tuberculous pulmonary lesion may be characterized by caseation necrosis that is absorbed only with difficulty over a long period. When bacilli within a predominantly necrotic lesion are suppressed, healing, in the strict pathologic sense of the word, does not occur unless absorption, evacuation, or organization follows. It is difficult for the host to dispose of the necrotic debris efficiently, and it may remain virtually without change over a long period of time. The inhibition of

therapy affords during the early weeks of treatment in almost every case. If resistant bacilli occur within the lesion after that, it is probable that favorable changes continue by virtue of the immunologic mechanisms of the host acting on bacilli, irrespective of their streptomycin-fastness or sensitivity, preventing their multiplication, and perhaps destroying many of them. It may be possible that the reduction of the total bacillary population of these lesions to numbers small enough to be dealt with effectively by the host, prevents the emergence of resistant strains. This would explain the low incidence of such bacilli actually observed in cases whose total pulmonary pathology consisted almost exclusively of scattered bronchopneumonic lesions.

Results in predominantly cavitary lesions seem also to be related more closely to the presence of necrotic foci than to the sensitivity of organisms. The converse of what was said above applies here. These lesions have relatively little tendency to heal during several months of therapy, whether or not bacilli are resistant to streptomycin. Thus, there is a low incidence of cavity closure even in those patients whose organisms retain sensitivity during 4 months of therapy. Many cavities are "lost to view," only to make their appearance when therapy is interrupted.

The higher incidence of resistant bacilli in patients whose total pulmonary lesion is largely cavitary is most probably related to the relative inability of necrotic foci to suppress bacillary multiplication. During the period of bacillary sensitivity that precedes the appearance of resistant organisms, the exudate, often relatively acellular, in such foci is apparently unable to destroy a few remaining bacilli, which presumably then become the progenitors of resistant strains.

The emphasis upon the pathologic features of lesions and the expected results of streptomycin therapy is not intended to minimize the importance of bacillary resistance. During the first course of therapy, both time and the potentialities of the tuberculous lesion determine the significance of resistant bacilli. In prenecrotic lesions, the rate of favorable change during the initial period of bacillary sensitivity tends partly to offset the potential disadvantages of the subsequent emergence of resistant bacilli; whereas in necrotic lesions, the maintenance of sensitivity throughout 4 months of therapy is but a limited therapeutic asset because of the inherent reluctance of the lesions to heal.

When the retreatment of patients in relapse is discussed, great stress will be placed upon the fact of bacillary resistance to streptomycin. It may be wondered how the retreatment of patients with resistant organisms differs from the continuation of streptomycin administration after bacilli become resistant. When a patient whose organisms are predominantly resistant suffers a new bronchogenic metastasis, it can be assumed that resistant

are evaluated in such divergent pathologic types, the results of streptomycin therapy will be markedly different, as is well known.

Correlations between therapeutic results, predominant pathologic types, and the emergence of resistant bacilli are shown by an analysis of forty-six patients who received streptomycin continuously for 4 months in doses of 1.0 and 0.5 gm daily (table 36). Each patient is listed according to his relative roentgenographic improvement in the group as a whole. The first patient in the table showed the greatest improvement during 4 months as measured by a comparison between the roentgenogram prior to therapy and that obtained at the end of therapy. The last patient improved least. There is a general correlation, first of all, between the types of pulmonary lesions and therapeutic results. Lesions consisting of a predominantly bronchopneumonic component showed the greatest improvement; those with a large cavitary component were not so favorably influenced. Moreover, patients who experienced the greatest improvement exhibited a lower incidence and lower ranges of bacillary resistance. Patients who improved least, on the other hand, revealed a higher incidence of resistant bacilli. Three factors, therefore, are correlated: therapeutic results, type of predominant pulmonary pathology, and incidence of resistant bacilli.

How these three factors are related to one another is a matter of the greatest importance for an understanding of the part played by resistant bacilli during streptomycin therapy. In considering the results of therapy, it would appear that the pathologic character of the lesion is of paramount importance. Recent bronchopneumonic lesions appear to respond well and to remain improved, irrespective of the sensitivity of bacilli to streptomycin *in vitro*. Approximately 80 per cent of patients with pulmonary tuberculosis who were treated for 4 months in the Veterans Administration Study Units (2) have shown varying grades of improvement roentgenographically In this investigation, the occurrence of recent bronchopneumonic lesions largely determined the selection of patients for therapy, and it must be assumed that the high incidence of improvement in the group as a whole represented favorable changes in this type of lesion. This point is of great significance when it is realized that *resistant* bacilli were recovered from the great majority of patients treated for that length of time. One is forced to conclude, therefore, that the emergence of such bacilli is not an invariably unfavorable influence upon the behavior of the recent bronchopneumonic lesion during a first course of therapy. This high rate of improvement associated with the high incidence or resistant bacilli has probably been the cause for doubt in the minds of some as to the clinical importance of the phenomenon of bacillary resistance.

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therapy affords during the early weeks of treatment in almost every case. If resistant bacilli occur within the lesion after that, it is probable that favorable changes continue by virtue of the immunologic mechanisms of the host acting on bacilli, irrespective of their streptomycin-fastness or sensitivity, preventing their multiplication, and perhaps destroying many of them. It may be possible that the reduction of the total bacillary population of these lesions to numbers small enough to be dealt with effectively by the host, prevents the emergence of resistant strains. This would explain the low incidence of such bacilli actually observed in cases whose total pulmonary pathology consisted almost exclusively of scattered bronchopneumonic lesions.

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TABLE 36

Correlations of therapeutic results, predominant pathology, and bacillary resistance in forty-six cases of pulmonary tuberculosis treated with streptomycin for 4 months

CASE NUMBER	ROENTGENOGRAM PRIOR TO THERAPY*		IN VITRO SENSITIVITY OF BACILLI†								
			During weeks of streptomycin therapy								After end of therapy
	Broncho-pneumonic component	Cavitary component	4	6	8	10	12	14	16	18	
1	++++	+	N	N	N	N	N	N	N	N	N
2	++++	0	S	S	S	N	N	N	N	N	N
3	++	+	N	N	N	N	N	N	N	N	N
4	++++	+	S	N	N	N	N	N	N	N	10–15
5	+++	+	S	S	S	S	S	S	S	10–15	10–15
6	++++	+	S	S	N	N	N	N	N	N	S
7	+++	0	S	N	N	N	N	N	N	N	N
8	+++	+	S	S	S	N	N	N	N	N	S
9	+++	++	S	S	S	S	S	S	S	S	S
10	++	++	S	S	S	N	N	N	N	>1000	>1000
11	++++	+	N	N	N	N	N	N	N	N	S
12	+++	++++	S	S	S	S	S	S	S	S	S
13	+	+++	S	S	S	S	S	S	S	S	S
14	++++	+	S	S	31–62	31–62	31–62	62–125	125–250	125–250	125–250
15	++	++	S	S	>1000	>1000	>1000	>1000	>1000	>1000	>1000
16	+++	++	S	S	31–62	31–62	31–62	62–125	>1000	>1000	>1000
17	++	++	S	S	S	S	S	S	S	S	S
18	++	0	S	S	S	S	S	S	S	S	S
19	++	++	S	S	10–15	10–15	10–15	15–31	15–31	31–62	31–62
20	+++	+++	S	S	S	S	S	S	S	S	S
21	++	+	S	S	S	S	S	S	S	S	S
22	++	+++	S	S	15–31	15–31	15–31	>1000	>1000	>1000	>1000

23	0	+	N	N	N	N	N	N	N	N	>1000
24	+++	0	S	S	N	N	N	N	N	N	N
25	0	+	N	N	N	N	N	N	N	N	>1000
26	+++	++	S	S	15–31	15–31	15–31	15–31	31–62	31–62	31–62
27	++	+++	S	N	N	N	>1000	>1000	>1000	>1000	>1000
28	++	+++	–	31–62	31–62	31–62	31–62	31–62	31–62	>1000	>1000
29	++	0	S	S	N	N	N	N	N	N	S
30	+	+++	S	S	S	–	62–125	31–62	62–125	62–125	62–125
31	+++	++	–	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
32	+	++	S	S	15–31	31–62	62–125	62–125	62–125	62–125	62–125
33	+	+++	S	S	S	>1000	>1000	>1000	>1000	>1000	>1000
34	++	+++	S	31–62	31–62	31–62	31–62	>1000	>1000	>1000	>1000
35	++	+	S	S	S	N	N	N	N	N	31–62
36	+	++	S	S	S	15–31	15–31	15–31	>1000	>1000	>1000
37	+	+++	S	S	S	S	S	S	S	>1000	>1000
38	+	++++	S	S	15–31	>1000	>1000	>1000	>1000	>1000	>1000
39	++	++++	S	S	>1000	>1000	>1000	>1000	>1000	>1000	>1000
40	+	++	S	S	>1000	>1000	>1000	>1000	>1000	>1000	>1000
41	+	++++	S	N	N	N	N	N	N	N	15–31
42	0	++	S	S	S	S	250–500	250–500	250–500	250–500	250–500
43	++	++++	S	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
44	+	+++	S	15–31	62–125	62–125	62–125	62–125	62–125	62–125	62–125
45	+	+++	10–15	10–15	>1000	>1000	>1000	>1000	>1000	>1000	>1000
46	+	++	31–62	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

* The extent of each component on the roentgenogram obtained prior to therapy is graded from 1 to 4+.

† In micrograms of streptomycin per milliliter of Tween-albumin medium. The first figure in each column represents the highest concentration of streptomycin that permitted growth of bacilli; the second figure, the lowest concentration that inhibited growth.

N—denotes negative sputum or gastric cultures.

S—denotes a sensitive strain which was inhibited by 10 0 μg of streptomycin per millimeter of medium.

bacilli are present within the new lesion. The pathologic character of the new focus is not distinctive and is, so far as is known, capable of complete resolution. Healing, however, must be brought about by the natural defense mechanisms of the host—attributes which are likely to be ineffectual, since, at some time in the past, the same patient showed limited ability to control his disease, and for that very reason became a candidate for streptomycin therapy. The importance of bacillary resistance here is precisely this: that bacilli within the new lesion cannot be quickly inhibited during the prenecrotic phase by the use of streptomycin; nor can bacilli be suppressed in the area which was the source of the metastasis, and further extensions of disease may occur.

In the case of continuous streptomycin administration to the patient whose organisms have become resistant, the situation is quite different. During the period of bacillary sensitivity early in the course of therapy, the inhibition of bacilli by streptomycin tends to prevent the progression of recent lesions to the necrotic phase. These gains are then maintained and furthered by innate capacity of the lesions to heal, whether or not bacilli become resistant. The distinction between these two situations is largely one of time.

Finally, the possibility that unfavorable changes occurring during bacillary resistance may be directly referable to the greater virulence of resistant bacilli should be mentioned. There is an occasional patient whose disease appears to progress especially rapidly during the latter part of therapy, after bacillary sensitivity has been lost. Steenken (3) has shown that guinea pigs inoculated with resistant organisms and treated with streptomycin died sooner than controls infected with similar strains. The fact that streptomycin-enhanced tubercle bacilli exist also contributes to the suspicion that continued therapy may be deterimental (4). Available clinical data, however, do not warrant the conclusion that the pathogenicity of resistant bacilli is increased in the presence of streptomycin. The point should receive further study

Symptomatology and the Sensitivity of Bacilli

Decrease in cough and sputum volume, a decline in fever, and gain in weight during the first 2 months of streptomycin therapy have been frequently observed. When therapy is continued for 4 months, symptomatic deterioration occurs in a significant proportion of patients. It is almost invariably associated with the appearance of resistant organisms *in vitro* The converse, however, is not necessarily true Patients who harbor resistant organisms may continue to improve and exhibit no symptomatic evidence of bacillary resistance

No generalizations can be made about the clinical significance of the association. Patients who have a recurrence of one or more symptoms may yet profit a great deal from therapy Much depends on the behavior of those lesions toward which therapy is directed. We have seen the lesions which prompted therapy continue to improve when the reappearance of symptoms, such as cough and increased sputum volume, was undoubtedly due to unfavorable changes in a large cavitary area. These changes were associated with the emergence of resistant bacilli. The correlation in time between unfavorable symptomatic changes and emergence of resistant bacilli is important in that it serves to emphasize the usefulness of laboratory methods in determining the sensitivity of organisms *in vivo*.

Cavitary Lesions and Bacillary Sensitivity

It has been frequently noted that cavities decrease in size during the first few weeks of streptomycin therapy. This favorable change appears to occur irrespective of the known duration of the lesion and is so commonly observed as to be expected in the great majority of patients treated. Simultaneous with the reduction in cavity size, resolution and better definition of the shadows constituting the so-called cavity wall may occur.

With the emergence of resistant bacilli *in vitro*, an appreciable proportion of cavities begin to enlarge, and some attain their former size. On the other hand, such enlargement, in our experience, is almost never observed during treatment when bacilli retain sensitivity. The temporal relationship between the unfavorable behavior of cavities and the emergence of resistant bacilli is so striking as to allow one to anticipate confidently the laboratory's demonstration of such changes in the sensitivity of bacilli *in vitro*. The correlation has validity irrespective of the mechanism responsible for cavity enlargement: whether it is due to changes in the lesion composing the cavity wall or to changes in the bronchus to the segment in which the cavity is located. It is unlikely that resistant bacilli are in themselves the direct causative factor. Rather, it is more probable that this type of predominantly necrotic lesion resumes its former potentialities when bacilli within it are no longer inhibited by streptomycin.

It is pertinent to inquire whether the relatively low incidence of cavity closure during streptomycin therapy is related to the phenomenon of bacillary resistance. This question cannot be satisfactorily answered at the moment, since administration of streptomycin over a long period results in a loss of bacillary sensitivity in the great majority of patients; and it is reasonable to suppose that long-continued bacillary suppression would be necessary to influence definitively this essentially necrotic lesion. It may be added, however, that the incidence of cavity closure is also low among these patients whose organisms retain sensitivity during 4 months of con-

tinuous therapy. It is probably that the character of the lesion is the more important factor in the failure of cavities to close completely in the relatively short time during which streptomycin is effective.

The increase in cavity size during treatment contemporaneously with the appearance of resistant bacilli *in vitro* is of variable clinical significance. In some instances the change is rather trivial and is significant only as evidence that resistant bacilli have developed in appreciable numbers. On the other hand, there are patients who seem to lose ground because of such changes. There is a time during therapy when cavities are at their smallest; and, in retrospect, it has appeared as though this would have been the most opportune time for the institution of collapse therapy, especially thoracoplasty.

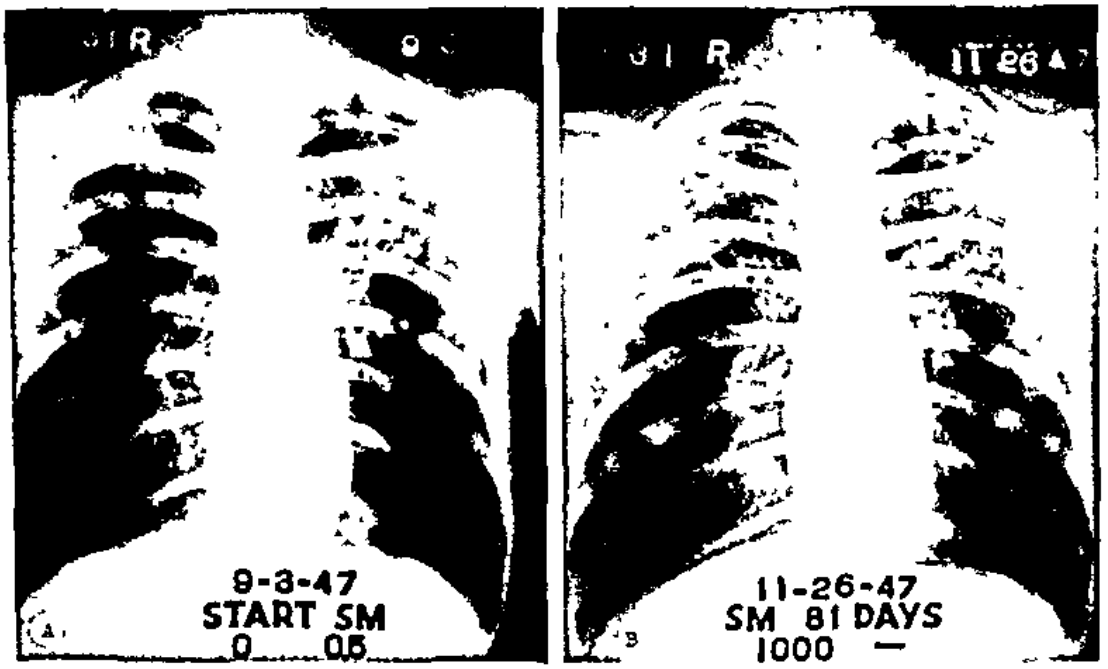
Although it may seem that the optimum time for thoracoplasty in these individuals has passed, the validity of this assumption must await further investigation. It is problematical whether patients are actually good candidates for this procedure because lesions appear roentgenographically feasible for thoracoplasty during or after streptomycin therapy. In the usual course of events, most patients become acceptable for thoracoplasty by virtue of their native resistance to tuberculosis. The very fact that they are able to attain relative stability indicates that immunological mechanisms are at least adequate to that degree. In many instances, lesions are quiescent, and surgical collapse measures are, in a sense, a method of controlling a potentially hazardous architectural defect, namely, cavitation. On the other hand, it is possible that lesions which appear to have become roentgenographically suitable for thoracoplasty because large cavities have become smaller and because collateral infiltration has largely resolved during streptomycin therapy, may not be definitely controlled by thoracoplasty. The native resistance of patients who have been selected for streptomycin therapy because lesions have progressed during a regimen of bed rest is, *in all probability, less than that of patients* who have the capacity to stabilize their disease. It is likely that this relative lack of native resistance to tuberculosis will manifest itself subsequently even though lesions have been enclosed within an anatomically adequate thoracoplasty. Studies that will contribute data toward an answer to this very important question are in progress (5).

Relapses and Bacillary Sensitivity

The unfavorable changes that are observed during and following streptomycin therapy may be of several kinds and of variable importance. Their temporal *relationship to bacillary resistance* appears to be unmistakable. As was mentioned previously, symptomatic relapse was noted frequently contemporaneously with, and following, the demonstration of

resistant bacilli in individual patients. In our experience, symptomatic deterioration in patients whose organisms retain sensitivity is a distinct rarity.

The enlargement of cavities observed during the development of bacillary resistance may be considered a variety of roentgenographic relapse. The incidence of this event is high, and, as mentioned in a previous paragraph, may or may not be therapeutically important. Local bronchogenic extentions of disease adjacent to enlarged cavities occur with less frequency. Such changes in the size of cavity and in the lung adjacent to cavity are frequently not recorded as relapses. This is largely a question of terminology.



first course of streptomycin per milliliter

If only bronchogenic metastases to parts of the lung which were previously uninvolved are considered as relapses, then their incidence during therapy is decidedly low. This type of relapse is illustrated in figure 60. In all probability, it is this variety of relapse that was recorded in the series of patients, reported in the Minutes of the Fifth Streptomycin Conference of the Veterans Administration (2). Here the incidence of relapse during therapy in two groups of patients, 375 treated with 1.8 gm daily, and 398 who received 1.0 gm daily, was 6.9 per cent and 9.5 per cent respectively. The infrequency of this type of relapse during therapy is, of course, of great significance, for patients who experience them are, in many instances, worse than prior to streptomycin therapy. In contrast, those who experi-

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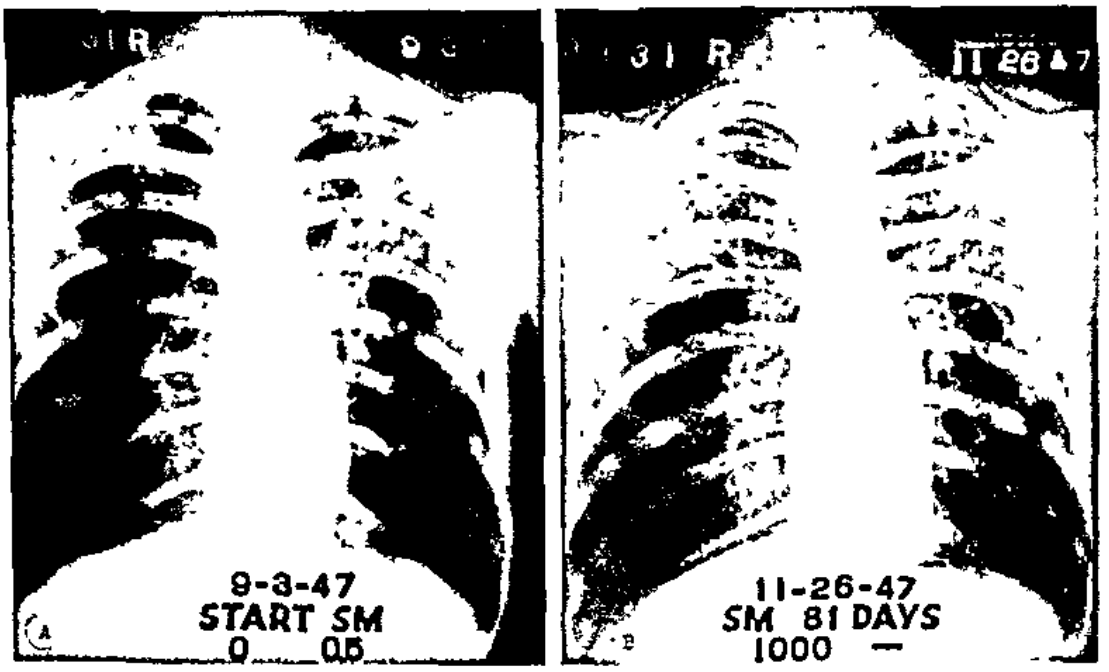

FIG. 60. A and B illustrate progression of disease during a first course of streptomycin. Organisms were resistant to over 1000 mg of streptomycin per milliliter of medium on 67th day.

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Relapses and Bacillary Sensitivity

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occurred in 22.8 per cent, and 77.2 per cent were unchanged or worse. Though data did not permit correlation between therapeutic efficacy and bacillary sensitivity in individual patients, it is, nevertheless, significant that the incidence of bacillary resistance in the group of patients from which the thirty-five were selected for retreatment was approximately 80 per cent. In the series of patients originally treated with 1.0 gm of streptomycin for 4 months, thirteen were subsequently retreated. Of these, 38.5 per cent improved, and 61.5 per cent were unchanged or worse. The incidence of bacillary resistance in the original group of patients was 65 per cent. The correspondence between the incidence of bacillary resistance in both series and the incidence of unfavorable results following retreatment appears to be significant.

Among the patients with pulmonary tuberculosis studied at Sunmount, nineteen were retreated because of relapses. Of these, fifteen had previously received continuous streptomycin for 4 months, and the duration of the first course had been 42 to 60 days in the remaining four. The results of retreatment, correlated with the degree of bacillary resistance, the duration of retreatment, and the daily dosage of streptomycin are shown in table 37.

It will be noted that those patients whose organisms were highly resistant *in vitro failed to respond favorably in every instance. The ineffectiveness* of retreatment was evidenced by continued progression of the lesions roentgenographically, by a lack of symptomatic improvement, continued loss of weight, and sustained fever. All eventually died of progressive tuberculosis. The roentgenographic progress of the disease in one such patient is illustrated in figure 61.

Patients whose organisms exhibited intermediate degrees of resistance *in vitro* showed varying degrees of improvement. Several, however, did not improve at all. One patient who was retreated twice is especially instructive. He is illustrated by cases 13 and 14, table 37. During the first period of retreatment, a right lower lobe cavity of moderate size was "lost to view," and sputum cultures were negative. Approximately 45 days later, the cavity again made its appearance, and a third course of streptomycin was begun. Bacilli obtained prior to their course were not inhibited by 15 μg of streptomycin per milliliter of medium. During this retreatment period the cavity not only failed to become smaller, but the patient suffered a bronchogenic metastasis to the base of the right lung (fig. 62)

Seven of eight patients (cases 12, 14, 15, 16, 17, 18, and 20, table 37) whose organisms were sensitive (that is, inhibited by 10 μg per milliliter of medium) improved upon retreatment. In several patients only slight roentgenographic improvement was noted during approximately 2 months

ence symptomatic relapse and unfavorable changes in and around areas of cavity formation may be greatly improved when roentgenograms prior to and at the end of therapy are compared.

It appears likely that the incidence of relapses of all types, symptomatic and roentgenographic, is associated with the presence or absence of necrotic foci and, more specifically, cavity formation—lesions that show a limited tendency to heal during bacillary inhibition by streptomycin. Correlations of predominantly cavitary disease, therapeutic results, and bacillary resistance were mentioned previously. It is reasonable to include relapses within these relationships.

The relapse rate following interruption of streptomycin therapy depends on the predominant type of pathology originally treated. The relationship to the sensitivity of bacilli is probably indirect. Patients who still have large areas of cavitation at the end of therapy have the greatest chance of relapse. These are the patients whose organisms are likely to have developed resistance during therapy. On the other hand, the frequency of relapse in patients whose organisms remain sensitive is certainly no less when areas of cavity formation persist after the interruption of therapy.

Results of Retreatment of Patients in Relapse and Sensitivity of Bacilli

Relapses may be expected following the interruption of any form of therapy that is not definitive for tuberculosis. It is pertinent to inquire, therefore, whether such relapses can be favorably influenced by a second course of streptomycin. Therapeutic results in animals inoculated with resistant bacilli and treated with streptomycin suggested that the retreatment of patients in relapse would not be particularly successful if bacilli were predominantly resistant (6, 7). This has been borne out by clinical experience Muschenheim and his associates (8) reported the results observed in seven patients with pulmonary tuberculosis who relapsed during and following the interruption of therapy In all seven, bacilli were predominantly resistant, and re-institution or continuation of therapy had no appreciable effect on the course of the disease, which, in all cases, terminated fatally. Fisher *et al* (9) retreated six relapsed patients when organisms were resistant to streptomycin and noted no evidence of further benefit.

Data from the Minutes of the Fifth Streptomycin Conference (2) of the Veterans Administration also suggest that the results of the retreatment of relapses in pulmonary tuberculosis are generally unfavorable. Thirty-five patients who had received a first course of 1.8 gm of streptomycin daily for 4 months were retreated, presumably because of relapse. Improvement

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of retreatment, but after that, resolution proceeded to an excellent final result. One such patient is illustrated in figure 63. It should be added

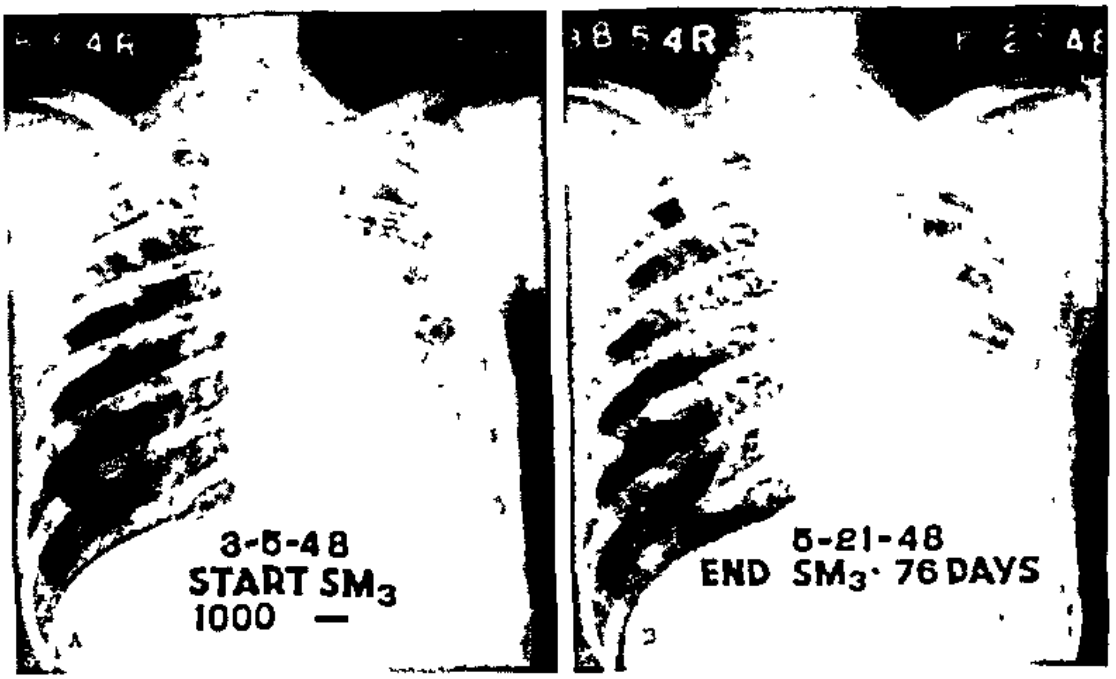

FIG. 61. A and B (case 3, table 37) illustrate the progression of disease during the retreatment of a patient whose organisms were highly resistant *in vitro*.

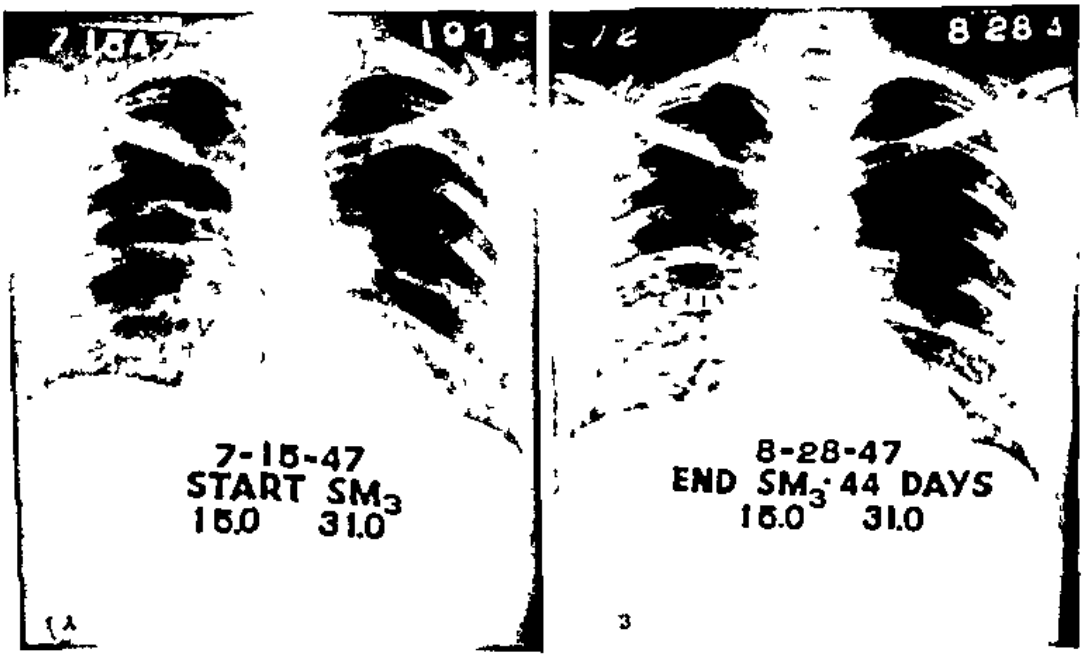

FIG. 62. A and B (case 13, table 37) illustrate the progression of lesion during the retreatment of a patient whose organisms were in an intermediate range of resistance to streptomycin.

that the extent of the relapse in most patients whose organisms were sensitive was appreciably less than in patients who harbored highly resistant organisms. This factor appeared to be related to the type of pathology

TABLE 37

Results of retreatment of patients with pulmonary tuberculosis in relapse

CASE NUMBER	SENSITIVITY OF BACILLI*		DURATION OF RETREATMENT	DAILY DOSAGE OF STREPTOMYCIN	REMARKS
	Prior to retreatment	End of retreatment			
			days	*gm*	
1	>1000	>1000	31	1.8	Died 4 days after end of retreatment
2	>1000	>1000	120	1.0	Unimproved. Died 137 days after retreatment.
3	>1000	>1000	77	1.0	Unimproved. Died 44 days after retreatment.
4	>1000	>1000	42	1 0	Unimproved. Died 59 days after end of retreatment
5	>1000	>1000	70	1 0	Unimproved Died 6 months after end of retreatment.
6	>1000	>1000	33	1 0	Unimproved. Died during retreatment.
7	61–125	125–250	118	1.8	Slightly improved at first. Progression of disease during retreatment. Died 1 year later.
8	15–31	31–62	120	1.0	Slightly improved at first Progressed during retreatment. Died 74 days after retreatment
9	10–15	10–15	42	1.0	Moderate improvement. Thoracoplasty after conclusion of retreatment.
10	10–15	10–15	60	1.0	Excellent result
11	10–15	15–31	60	1.0	Moderate improvement at end of retreatment. Excellent resolution thereafter
12	5–10	15–31	42	1 0	Slight to moderate improvement Relapse after retreatment
13	15–31	15–31	44	1 8	Progression of disease during retreatment
14	0 0–0 5	Negative culture	84	1 8	Moderate improvement. Relapsed. Retreated again (case 13).
15	0 0–0 5	1 0	60	1 8	Moderate improvement. Relapsed again.
16	2 5–5 0	2 5–5 0	42	1 0	Slight improvement at end of retreatment. Excellent improvement thereafter
17	0 5–1 0	1 0–2 5	42	1.8	Excellent result Followed by pneumothorax.
18	1 0–2 5	Negative culture	42	1 0	Moderate improvement.
19	1 0–2 5	15–31	120	1 0	Progressive disease during retreatment Died 44 days after retreatment
20	2 5–5 0	15–31	140	1.0	Excellent result Thoracoplasty. Died 1 year later of fatal hemorrhage.

* In micrograms of streptomycin per milliliter of Tween-albumin medium. The first figure in each column represents the highest concentration of streptomycin that permitted growth of bacilli, the second figure, the lowest concentration that inhibited growth.

originally treated with streptomycin. That is to say, lesions that were not characterized by large areas of cavity formation improved most during the first course of streptomycin therapy, the bacilli tended to remain sensitive, and relapses, when they occurred, were in general less extensive.

One patient (case 19, table 37; fig. 64) whose organisms were predominantly sensitive at the start of retreatment progressed unfavorably though bacilli attained but a low range of resistance. This result was in great contrast to that obtained in another patient, the sensitivity of whose organisms was almost exactly similar (case 20, table 37). The dissimilar therapeutic results in these individuals were probably due to such variables as

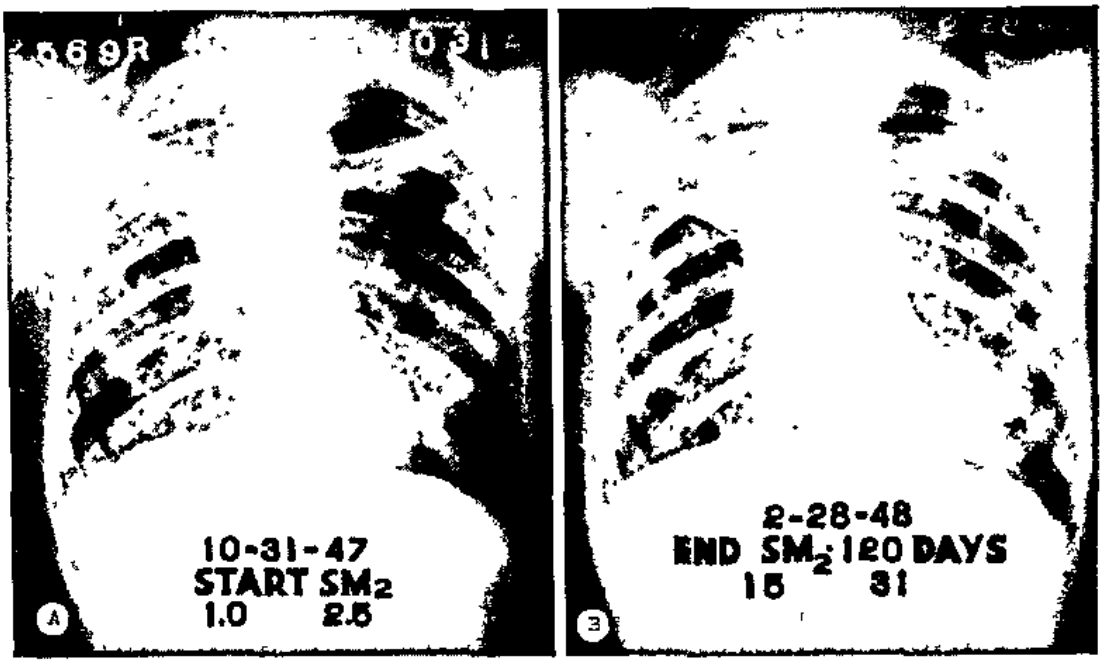

FIG. 64. A and B (case 19, table 37) illustrate the progression of disease in a patient who received a second course of streptomycin at a time when bacilli were predominantly sensitive. Note the intermediate range of resistance of bacilli at end of retreament.

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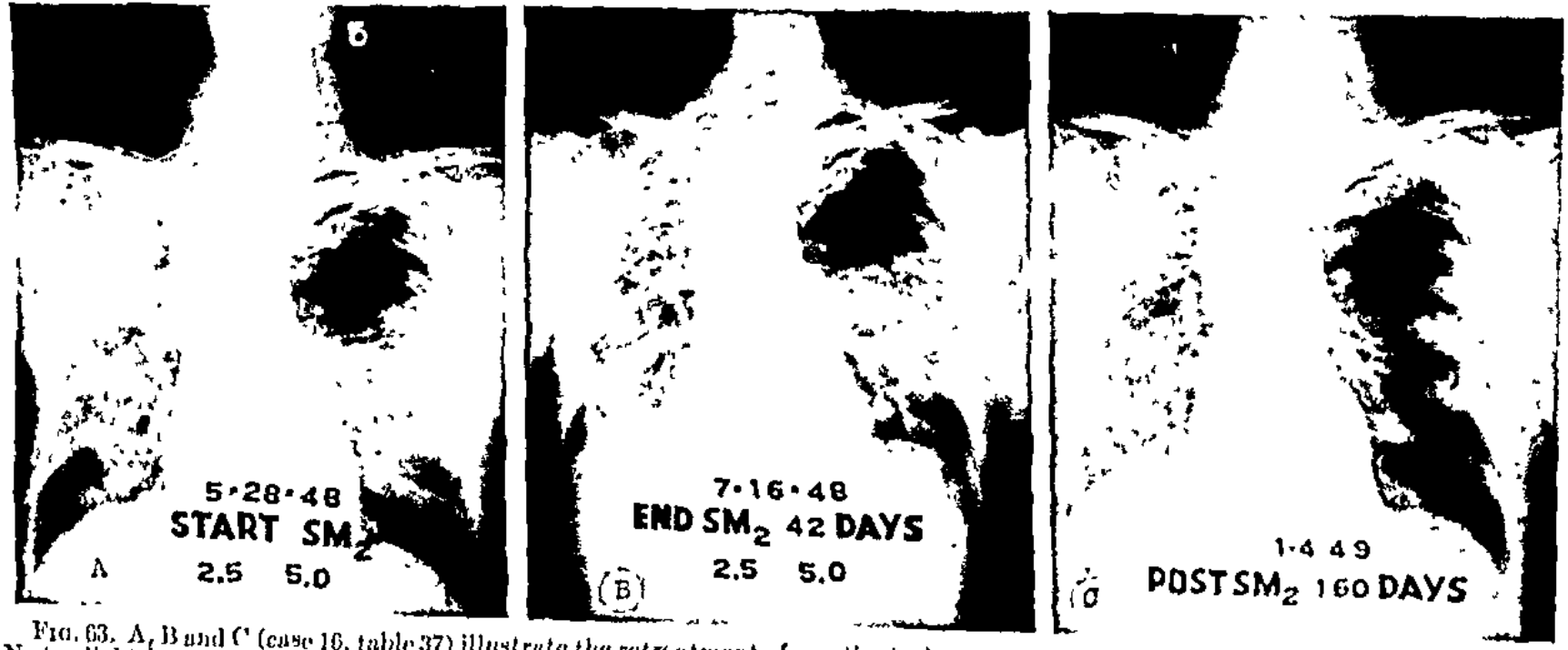
The sensitivity of bacilli recovered from these patients was determined in liquid synthetic media. This method does not reveal the relative proportions of sensitive and resistant organisms. Unpublished data, however, indicate an excellent relationship between the ranges of resistance obtained with this medium and the distribution of sensitive and resistant organisms as demonstrated by the use of solid media in which varying concentrations



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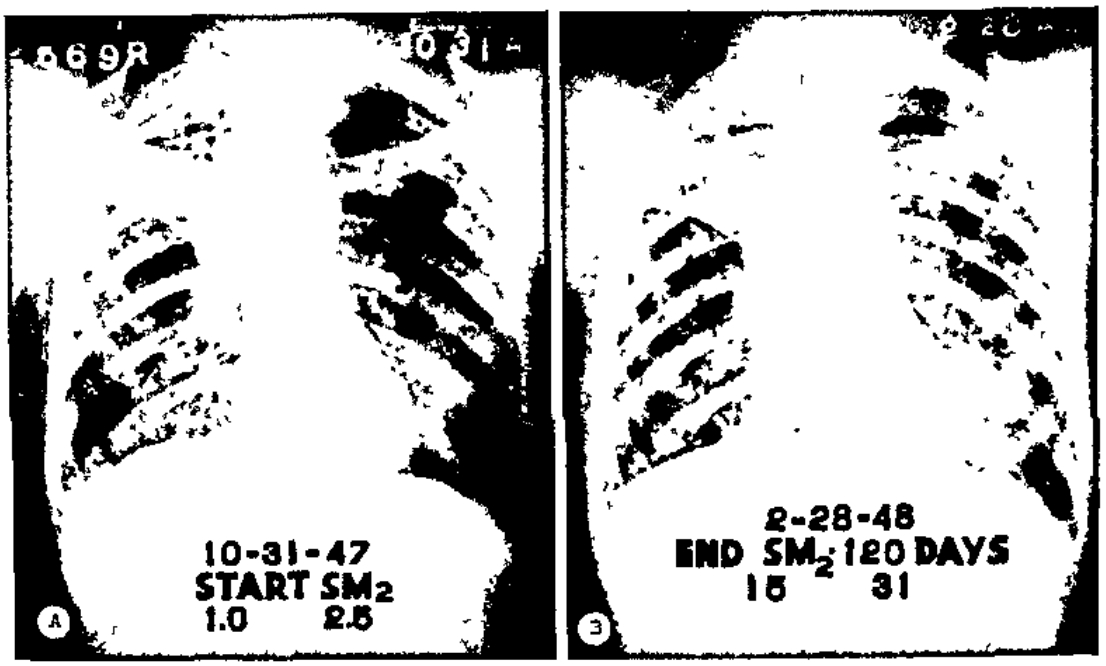

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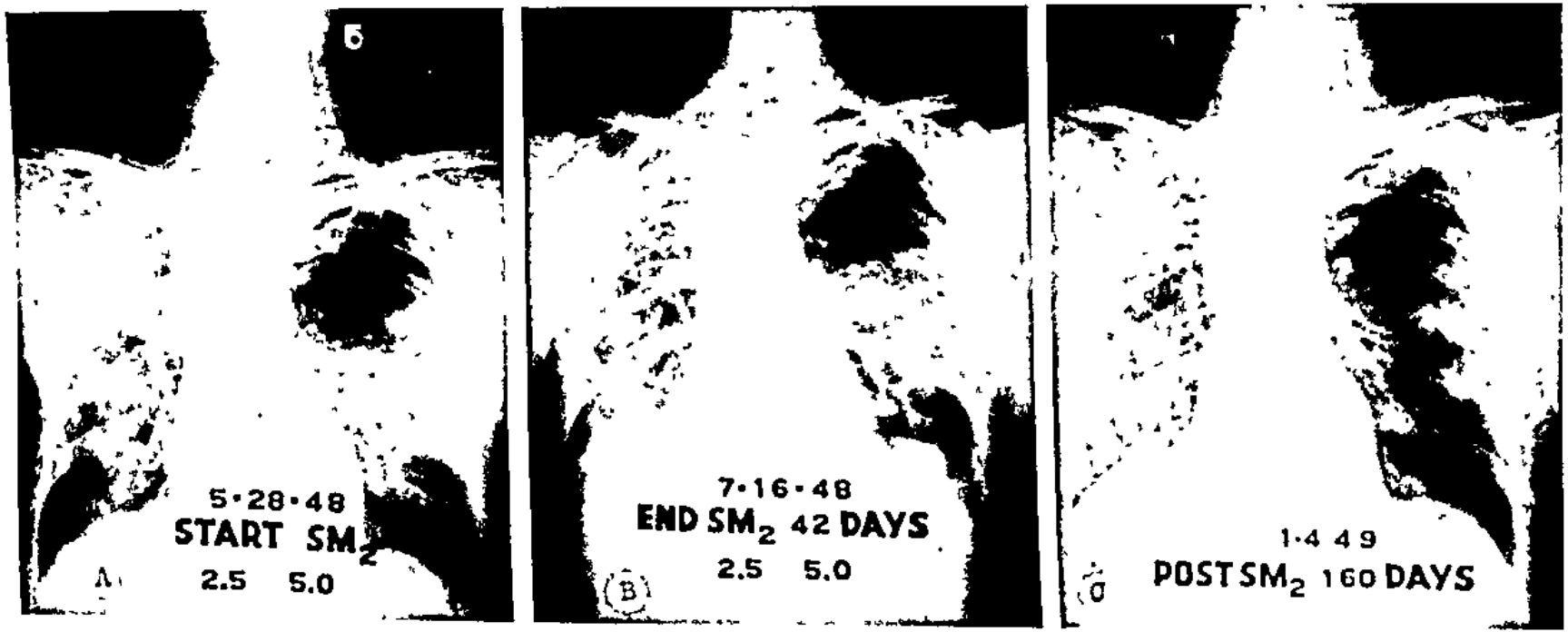

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originally treated with streptomycin. That is to say, lesions that were not characterized by large areas of cavity formation improved most during the first course of streptomycin therapy, the bacilli tended to remain sensitive, and relapses, when they occurred, were in general less extensive.

One patient (case 19, table 37; fig. 64) whose organisms were predominantly sensitive at the start of retreatment progressed unfavorably though bacilli attained but a low range of resistance. This result was in great contrast to that obtained in another patient, the sensitivity of whose organisms was almost exactly similar (case 20, table 37). The dissimilar therapeutic results in these individuals were probably due to such variables as

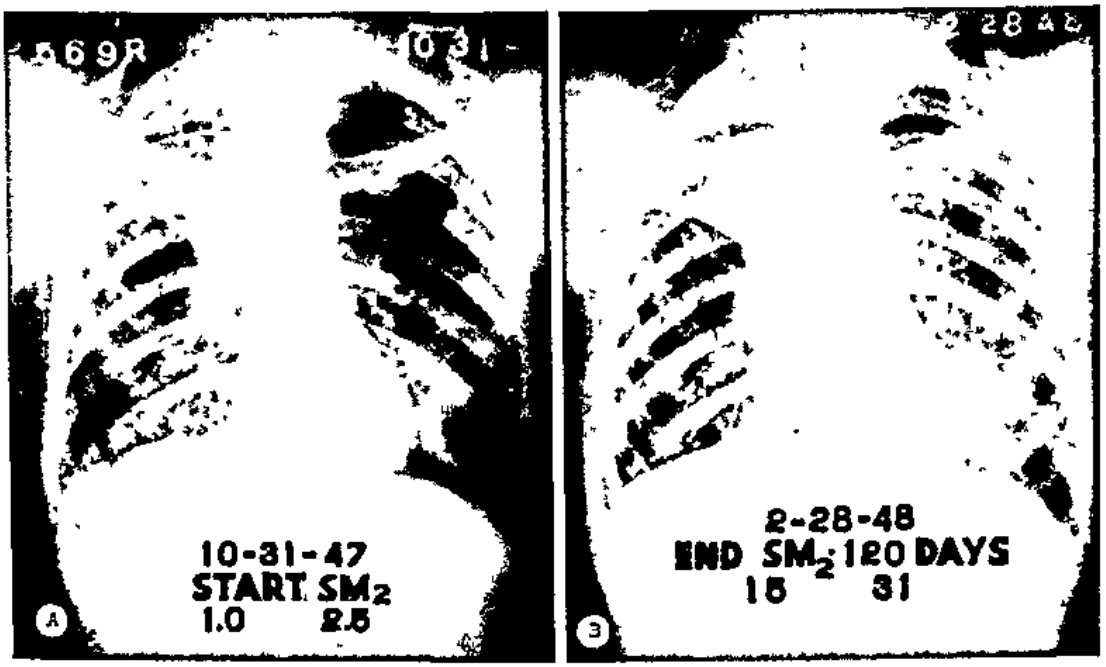

FIG. 64. ... who receive ... nantly sens ... treament.

the proportions of resistant and sensitive strains within their total bacillary population, the absolute number of organisms, the site of the bronchogenic metastases constituting the relapse, and the native resistance of each to tuberculosis. It is significant that in the case of patient 20 the new disease for which retreatment was instituted was at the base of the lung—a site which favors resolution.

The sensitivity of bacilli recovered from these patients was determined in liquid synthetic media. This method does not reveal the relative proportions of sensitive and resistant organisms. Unpublished data, however, indicate an excellent relationship between the ranges of resistance obtained with this medium and the distribution of sensitive and resistant organisms as demonstrated by the use of solid media in which varying concentrations

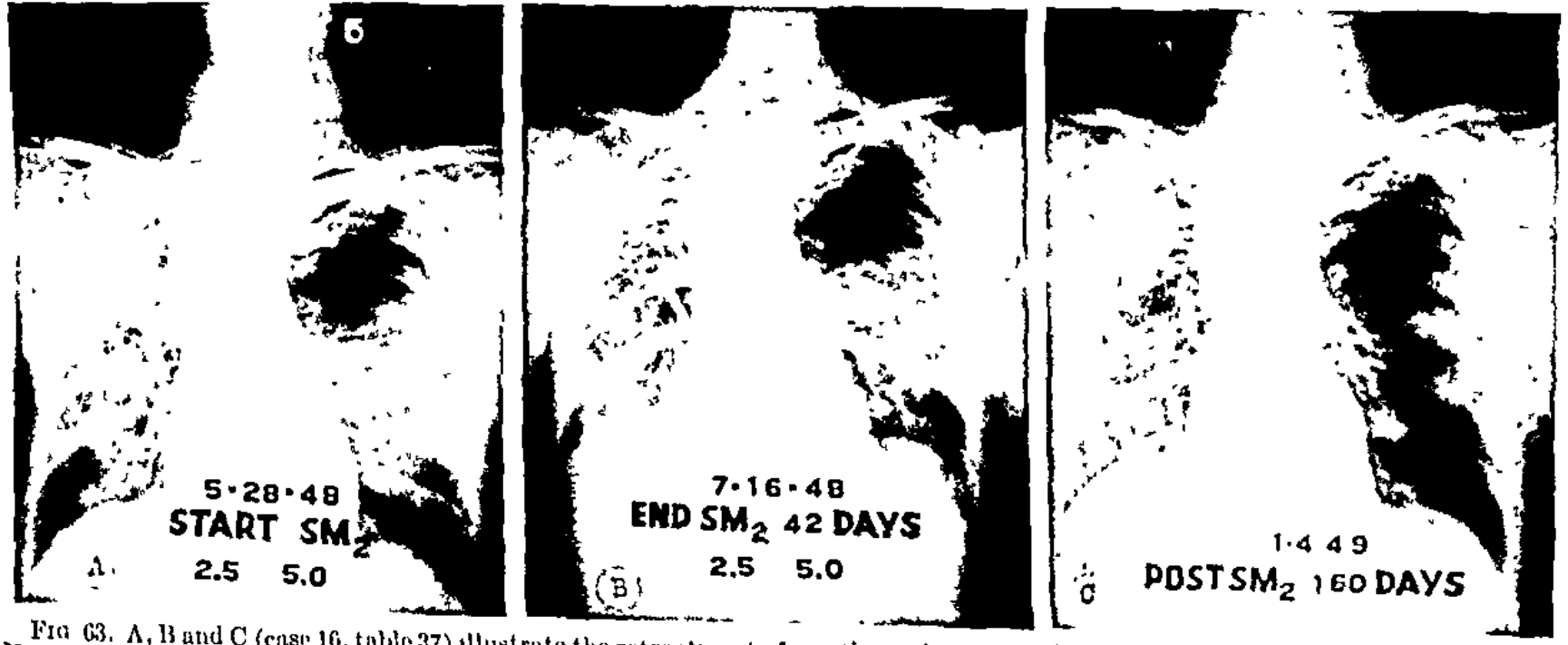

FIG 63. A, B and C (case 16, table 37) illustrate the retreatment of a patient whose organisms were predominantly sensitive *in vitro*. Note slight improvement at the end of retreatment, and *progressive resolution* thereafter.

Patients whose bacilli become highly resistant to streptomycin early in the course of 4 months of streptomycin therapy improve less than those whose organisms retain sensitivity *in vitro*. The predominant pathologic types of lesions, incidence of bacillary sensitivity, and therapeutic results show a general correlation.

Patients whose organisms become highly resistant during a first course of streptomycin do not respond favorably when retreated during relapse. Patients whose organisms are in the intermediate ranges of resistance may or may not be benefited. Those whose organisms are sensitive characteristically improve during retreatment.

The importance of the development of bacillary resistance to streptomycin cannot be overemphasized. The disadvantages of the loss of bacillary sensitivity to streptomycin are most readily appreciated by the results of the retreatment of patients in relapse

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of streptomycin are incorporated. These data indicate that when a culture includes organisms resistant to high concentrations of streptomycin, a very great proportion of the total culture is highly resistant. On the other hand, cultures that show intermediate ranges of resistance in liquid media (such as growth in 15 μg/ml and inhibition by 31 μg/ml) include a large number of sensitive bacilli and a variable proportion of intermediate and highly resistant bacilli. This newer method of testing the sensitivity of bacilli will undoubtedly help to explain why individual patients whose organisms are in the intermediate ranges of resistance may or may not respond favorably to a second course of streptomycin.

The factor of relapsing disease must be emphasized. The patient who relapses indicates the need for additional measures and demonstrates best the efficacy of subsequent therapy, in this instance, streptomycin. The response of patients who receive retreatment during a period of relative quiescence is almost impossible to evaluate and may obscure the significance of bacillary resistance.

The ineffectiveness of streptomycin in the retreatment of patients who harbor highly resistant organisms stands out as the major limitation of this tuberculostatic agent. The fact is of overwhelming significance when it is realized that most patients develop resistant organisms after but 4 months

[illegible]

streptomycin for the treatment of pulmonary tuberculosis suggests three general principles which should govern its use: First, streptomycin is highly inadvisable in the treatment of those patients whose disease can be controlled without great risk by conventional types of therapy. Secondly, when streptomycin is deemed necessary for the treatment of progressive pulmonary lesions, the duration of therapy should be such as to prevent a high incidence of bacillary resistance. The shorter courses of therapy, now advocated, and interrupted therapy, now being investigated, are designed to this end. And finally, it appears reasonable to institute collapse therapy during or soon after the cessation of therapy in order to consolidate whatever gains have been made This approach should be attempted, since the persistence of cavities appears to be largely responsible for relapses.

Summary

The sensitivity of tubercle bacilli to streptomycin *in vitro* accurately reflects the sensitivity of organisms *in vivo*. There are significant correlations between the sensitivity of bacilli and symptomatic and roentgenographic changes observed during therapy.

4. Long bones, mastoids, and middle ear
5. Meninges
6. Traumatic wounds and burns

Problems of Evaluation of Drug Therapy in Septicemia

A definitive sign of septicemia is culture proved bacteremia. Serial blood cultures following administration of the antibiotic are a key to the effectiveness of treatment. Blood cultures, however, cannot be used exclusively in the evaluation of treatment, because of limitations: (a) the number of such cultures before and after the onset of therapy is usually too few; (b) false "positives" due to contaminants, and false "negatives" due to bacteriostasis from circulating antibiotic or to cultures taken at intervals between septic showers, may give erroneous information of the role played by the therapeutic agent.

TABLE 38

*Sensitivity of gram-positive cocci to streptomycin and penicillin**
(Filter-disc agar-plate test)

	NUMBER OF CASES	PENICILLIN-RESISTANT STREPTOMYCIN-SENSITIVE	PENICILLIN-RESISTANT
Hemolytic *S aureus*	323	67 (23%)	40 (60%)
Gamma-Hemolytic Streptococci	54	25 (46%)	8 (32%)
Beta-Hemolytic Streptococci	108	15 (14%)	2 (13%)
Alpha-Hemolytic Streptococci	39	16 (41%)	1 (7%)

* Data compiled from Surgical Research Unit, Bacteriology Section, Brooke General Hospital, Fort Sam Houston, Texas.

After establishment of bacteremia by blood culture, the relation of changes in fever pattern and toxic manifestations to administration of drug helps evaluate the therapeutic agent. It must, however, always be kept in mind that the response of the septicemia to antibiotics may be independent of the effect on the underlying foci. Surgical or spontaneous drainage of the primary foci may reduce the numbers of bacteria entering the circulation so that humoral antibodies and phagocytosis can overcome the septicemia.

Classification of Results of Therapy of Septicemia

Good indicates that the patient was cured of his septicemia and that streptomycin played a significant part in the cure. *Poor* designates those cases in which septicemia was not cured while streptomycin was being administered. *Doubtful* cases are those which recovered from the septicemia

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CHAPTER 21
BACTEREMIA

DEFINITION

Bacteremia is invasion of the circulating blood by bacteria. Microorganisms may escape from any type of primary infection into neighboring veins, then circulate until taken up by hepatic, splenic, or lymphatic filters. Septicemia is toxemia from bacterial invasion of the circulating blood from a suppurating focus. Bacteremia and septicemia begin with circulating organisms and end when the bacteria no longer emerge continuously or intermittently into the circulating blood.

ETIOLOGY

Gram-positive cocci are the most frequent cause of septicemia. Most are penicillin-sensitive (table 38). Penicillin-fast strains, however, occur naturally while others may develop as a result of inadequate therapy. The tendency to develop penicillin-resistance is, in our experience, according to the following descending order: *S. fecalis*, *S. viridans*, *S. aureus*, and *S. hemolyticus*. Need for an alternative drug to combat penicillin-resistant cocci is obvious. Septicemia is due, less commonly, to gram-negative organisms, especially *E. coli*, *A. aerogenes* group, *Pseudomonas*, *Proteus*, and *Bacteroides*. Excluded from this discussion are infections by *Brucella*, *Pasteurella*, *Salmonella*, and *Hemophilus*, which are discussed elsewhere.

PATHOGENESIS

Gram-negative bacteria dominate the fecal flora and are commonly present in water and soil. Gram-negative organisms, therefore, may gain entrance into the blood stream from almost any focus in the body, but for practical purposes the following common sites of infection may be listed:

1. Gastro-intestinal tract, especially in infants and the severely debilitated, e.g. antemortum
2. Peritoneal cavity
3. Genito-urinary system

[1] Chief of the Surgical Research Unit, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas

gram-negative organisms. At this writing, results of therapy of a fairly large number of streptomycin-treated cases have been reported. Table 40 represents a total of cases of bacteremia in which streptomycin has been used. Cases of bacteremia associated with subacute bacterial endocarditis are included in this figure. In the 170 cases reported, a good response was obtained in 68 per cent and a doubtful response in an additional 6 per cent.

TABLE 40

Response of bacteremia to streptomycin therapy

INFECTING ORGANISM	NUMBER OF CASES	RESPONSE TO THERAPY		
		Good	Doubtful	Poor
E. coli	53	38	3	12
A. aerogenes . . .	12	8	1	3
E. coli and *A. aerogenes*	1			1
Kl. pneumonia	8	7		1
Pr. vulgaris . . .	7	7		
Ps. aeruginosa	17	10		7
Ps. aeruginosa and *A. acrogenes* .	1	1		
Ps. aeruginosa, *A. aerogenes*, and *E. coli*	1			1
A. fecalis .	2	1	1	
Neisseria	2	1		1
Bacteroides	2	1		1
A aerogenes and *Pr. vulgaris*	1	1		
Gram-negative, unidentified	8	5	1	2
E. coli and *S. fecalis*	1	1		
S. fecalis	8	7		1
S viridans	11	7		4
Streptococcus, unidentified	7	3		4
Staphylococcus	24	14	3	7
Corynebacterium	1	1		
B anthracis	1	1		
Spirillum	1	1		
Gram-positive, unidentified	1	1		
Total	170	116 68%	9 6%	45 26%

These figures represent a cross section of results obtained by a large number of investigators (8–28) and include cases treated with varying doses, at different stages of the disease and at times under adverse conditions. In any event it now appears that the recovery rate from bacteremia due to gram-negative organisms has increased from about 40 per cent before sulfonamide therapy to 75 per cent with streptomycin therapy. Thus it would appear that under early adequate treatment with streptomycin, good re-

while streptomycin was administered but in which the part played by streptomycin was difficult to evaluate.

Results of Therapy

Infections complicated by bacteremia have always been grave. An estimate of the mortality from gram-negative bacillary bacteremias before sulfonamide therapy can be gained from representative experiences listed in table 39. Thus *recovery in no instance exceeded 66 per cent (E. coli)*, while the overall mortality was about 60 per cent. The mortality from staphylococcal, streptococcal, and pneumonococcal septicemia in that era was even higher (7), ranging between 65 per cent and 85 per cent. After introduction of sulfonamides, reduction in mortality was most striking with the hemolytic streptococcal infections, but gratifying results were also obtained with *E. coli* and pneumococcal infections. In these conditions, sulfonamides reduced the mortality perhaps by three-quarters. Staphylococ-

TABLE 39

Mortality from gram-negative septicemia before sulfonamides

ETIOLOGY	NUMBER OF CASES	MORTALITY	REFERENCES
		per cent	
E coli ..	47	34	*Felty (1)*; Herrell (2)
Klebsiella .	43	86	Bullowa (3); Baehr (4)
Proteus	18	64	Abrams (5)
Pseudomonas	—	"Very high"	Stanley (6)
Bacteroides	8	10	Herrell (2)

cal sepsis was not so effectively overcome and continued to be a problem until the advent of penicillin Sulfonamides have the following salient

of protein degradation

Penicillin is a far more effective agent than sulfonamides for treatment of gram-positive bacteremia (7). Its effects in streptococcal, pneumonococcal, and staphylococcal bacteremia are often dramatic, and the chances of cure with vigorous early treatment exceed 75 per cent. Penicillin therapy in subacute bacterial endocarditis caused by susceptible organisms has also been gratifying. It has the advantage of low toxicity to the host and is not likely to result in a dominance of infections by resistant bacterial strains. Its action on gram-negative bacteria, however, is comparatively feeble.

Immediately after streptomycin became available, it was evident that there was a new and effective weapon for treatment of bacteremia from

ported subacute bacterial endocarditis treated with penicillin. Sixty-eight per cent of the patients were regarded cured or in remission. When cases were excluded because of what is currently regarded as too small dosage after sterilization of the blood, the recovery rate was 78 per cent. Similar experience with streptomycin at this time is limited. Of thirty-two known cases (19–28), sixteen (44 per cent) had a good response, fourteen had a poor response, and in two cases the effect was doubtful. In twenty-three of these cases, streptomycin only was administered. In the remaining nine, penicillin and/or sulfadiazine was administered concurrently with

TABLE 41

Streptomycin therapy in bacterial endocarditis

INFECTING ORGANISM	NUMBER OF CASES	STREPTOMYCIN ALONE			STREPTOMYCIN WITH PENICILLIN			STREPTOMYCIN WITH PENICILLIN AND SULFADIAZINE			STREPTOMYCIN WITH SULFADIAZINE		
		Good	Doubtful	Poor	Good	Doubtful	Poor	Good	Doubtful	Poor	Good	Doubtful	Poor
E. coli	1			1									
A. aerogenes	1			1									
Ps aeruginosa	3			2			1						
Neisseria	1	1											
Gram-negative, unidentified	4		1	1		1					1		
S. fecalis	8	2		5	1								
S. viridans	11	5		2	2		1	1					
S. aureus	2	1						1					
Corynebacterium	1	1											
Totals	32	10	1	12	3	1	2	2	0	0	1	0	0
Good response	16	10			3			2			1		
Doubtful response			1			1							
Poor response				12			2						

streptomycin (table 41). Most of the patients had been treated previously with penicillin or sulfonamides without success. This series, though small, suggests that streptomycin is indicated in: (a) subacute endocarditis caused by *S faecalis* or by other streptococci refractory to large doses of penicillin, and (b) the rare case of infection of gram-negative etiology where streptomycin susceptibility is demonstrated. Courses of therapy in this condition must frequently be continued for 21 or more days; treatment must always be continued for at last 48 hours after the temperature has returned to normal. A certain proportion of patients may therefore be expected to show vestibular damage as the result of streptomycin toxicity. The risk must

sults may be expected in the majority of cases caused by susceptible organisms.

It is concluded from these data that streptomycin is indicated in all cases of bacteremia caused by gram-negative organisms. It can often be expected to clear the blood from infected organisms and influence favorably the local lesion. Where surgery is necessary, streptomycin therapy provides a protective umbrella during direct attack on the underlying focus.

The series of gram-positive streptomycin-treated bacteremias, though small, is significant, because it represents, in the main, failures with penicillin. Of fifty-five such cases, forty-one (74 per cent) responded adequately to streptomycin. Bacteremias caused by *S. faecalis* responded best to streptomycin, eight cases showing a good response and one case a poor response. Of eighteen cases caused by other streptococci, ten (56 per cent) showed a good response and eight (44 per cent) indicated no benefit. Of twenty-four cases of staphylococcic septicemia, 58 per cent responded well to streptomycin, 29 per cent did poorly, and the effect of therapy was doubtful in 13 per cent. One case each of *B. anthrax*, *Corynebacterium*, and a *Spirillum* septicemia is reported as benefited by streptomycin therapy. An important indication for streptomycin, therefore, is gram-positive bacteremia caused by moderately penicillin-resistant but streptomycin-sensitive organisms in which the outcome is in doubt after 48 hours of therapy with optimal doses of penicillin. Penicillin and streptomycin should be used simultaneously in these cases, to provide maximum coverage. Where *in vitro* testing shows that the organism will grow in concentrations of penicillin not possible in the blood serum, but will show inhibition by concentrations possible by parenteral administration of streptomycin, the two drugs should still be used concurrently, to provide maximum coverage.

Our laboratory studies (29) show that optimal bacteriostatic effects *in vitro* are obtained on aerobic bacteria with combinations of streptomycin, penicillin, and sulfadiazine Significant corroborative clinical data are not available at this time.

Causes of Failure of Treatment of Septicemia

Failures in these cases occurred, for the most part, for the reasons of failure of chemotherapy of any infection. They include: (a) tardy treatment; (b) complicating acute vegetative endocarditis, (c) development of streptomycin resistance by the organisms, (d) inadequate management, during therapy, of the primary focus of infection, (e) inadequate dosage; and (f) development of toxicity to streptomycin during therapy.

Subacute Bacterial Endocarditis

Zeller, Hirsh, Dowling, Hussey, and Robinson (21) recently reviewed all available British and American contributions and found 556 cases of re-

ported subacute bacterial endocarditis treated with penicillin. Sixty-eight per cent of the patients were regarded cured or in remission. When cases were excluded because of what is currently regarded as too small dosage after sterilization of the blood, the recovery rate was 78 per cent. Similar experience with streptomycin at this time is limited. Of thirty-two known cases (19–28), sixteen (44 per cent) had a good response, fourteen had a poor response, and in two cases the effect was doubtful. In twenty-three of these cases, streptomycin only was administered. In the remaining nine, penicillin and/or sulfadiazine was administered concurrently with

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S. viridans	11	5		2	2		1	1					
S aureus	2	1						1					
Corynebacterium	1	1											
Totals	32	10	1	12	3	1	2	2	0	0	1	0	0
Good response	16	10			3			2			1		
Doubtful response			1			1							
Poor response				12			2						

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be accepted, however, when subacute bacterial endocarditis is not amenable to other forms of available antibiotic or chemotherapy. This risk may now be minimized by the use of dihydrostreptomycin.

Comment

Streptomycin therapy will be successful in bacteremia only under certain requisites: 1. The organism must be sensitive to the drug; 2. Accessible foci of infection must be adequately drained; 3. The dosage of streptomycin must be at least 2 gm a day, and the intervals between doses must be so spaced as to provide for bacteriostatic blood levels; 4. Treatment must be continued for a sufficiently long time; 5. Acute endocarditis, which is uniformly fatal, must not be present.

Streptomycin therapy seems warranted in the case of bacteremia caused by gram-positive cocci when a favorable response to maximum dosages of penicillin has not been obtained. The combination of subinhibitory concentrations of streptomycin and penicillin with sulfadiazine is likely to be useful because of the additive effects thus obtained. Streptomycin is clearly indicated in bacteremias caused by *S. faecalis*, which is ordinarily refractory to large dosages of penicillin. In this and other penicillin-refractory bacteremias, penicillin and streptomycin in combination may be lifesaving.

Streptomycin is also indicated in the therapy of subacute bacterial endocarditis caused by penicillin-fast nonhemolytic streptococci and susceptible gram-negative bacilli.

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Incidence of meningitis due to gram-negative bacilli and Mycobacterium tuberculosis

Compiled by Paine, Murray, Seeler, and Finland (1); our experience has been added

AUTHORS	CASES OF MENINGITIS	ORGANISMS			
		H. influenzae	Coliform organisms	Other gram-negative bacilli*	*Mycobacterium tuberculosis*
	All ages				
Neal (1935)[1]†	3178	142	8	11	986
Tripoli (1936)[2]†	468	20		4	51
Rhoads *et al.* (1940)[3]	459	29			158
Ferguson and Barr (1941)[4]	72	2	1		6
Rhoads (1947)[5]	550	15	2		16
Brainerd and Bradley (1947)[6]	265	7	5		14
Total ...	4992	215 (4.3%)	16 (0.32%)	15 (0.30%)	1231 (24.7%)
Columbia-Presbyterian Medical Center (1938–1948)	436	110 (25%)	7 (0.02%)	3 (0.01%)	79 (17%)
	Infants and children				
Fothergill and Sweet (1933)[7]	705	78	9	1	290
Lindsay *et al.* (1940)[8]	642	100		2	205
Silverthorne (1943)[9]	1100	153			368
Neal (1935)[1] (under 3 years)	1077	92	6	4	440
Total...	3524	423 (12.0%)	15 (0.43%)	7 (0.20%)	1303 (37.0%)
	Autopsies				
Keefer (1941)[10]	83	9			9
Hertzog (1945)[11]	377 (149)‡	43 (39)‡	10 (9)‡	1	56 (16)‡
Pote and Courville ⁂ (1945)[12]	100 ⁂	7**	4**	2	2
Total. ...	560	59 (10.5%)	14 (2.50%)	3 (0.41%)	67 (12.0%)

* Includes *Kl. pneumoniae*, *Ps. aeruginosa*, typhoid-dysentery organisms, *Pr. morganii*, and others.
† References 1 through 12 supplied by Paine, T. F., Murray, R., Seeler, A. O., and Finland, M. (1).
‡ Number of patients under 3 years old.
⁂ Meningitis complicating disease of the nose, accessory nasal sinuses, or ears.
** Certain of these were mixed infections, including one case with *A. aerogenes* and a streptococcus.

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Department of Pediatrics, Columbia University

CHAPTER 22

NONTUBERCULOUS MENINGITIS

Streptomycin has proved to be the most valuable single therapeutic agent for treatment of meningitis caused by gram-negative bacilli. Although this antibiotic has been shown to be active *in vitro* against a number of gram-positive coccal species which at times invade the meninges, other therapeutic agents are superior; only when emergence of resistance of the organism has changed the efficacy of penicillin is streptomycin indicated in meningitis caused by gram-positive cocci.

Though any one of the gram-negative bacilli may infect the meninges in any age group, most of them do so rarely. The relative importance of two of these varieties, *H. influenzae* and *E. coli*, compared with other species of this group is shown by their frequency of occurrence in table 42. It is apparent that *H. influenzae* meningitis is by far the most important member of meningeal infections caused by gram-negative bacilli and that *E. coli* ranks second. Among the rest of the group *Ps. aeruginosa*, *Kl. pneumoniae* (Friedländer's bacillus), Salmonellae, *Proteus*, *A. aerogenes*, and *A. faecalis* appear to occur most frequently.

The early contributions of Waksman and his associates suggested that all of these organisms exhibit a degree of sensitivity to streptomycin commensurate with therapeutic efficacy of this agent. Similar results were reported by other investigators Many of the gram-negative bacilli were also demonstrated to be susceptible to streptomycin *in vivo*. The results of even our early *in vitro* experiments (2) suggested, however, that gram-negative bacilli differ significantly in their sensitivity to streptomycin; some members withstood high concentrations of streptomycin when the following principles were applied to the test procedure: (a) the inoculum was large (3 million to 1,700 million) and therefore more representative of the population sizes in biologic fluids of patients; (b) the medium was solid and optimal (Levinthal agar), and (c) the incubation period was 48 hours. The last point was important, since the more resistant organisms grew more slowly.

therefore, to the observed conspicuous differences in therapeutic efficacy of streptomycin among infections caused by these species.

In an attempt to explore the causes for these differences two factors were studied for each of the eight species:

1. The frequency of occurrence of their resistant mutants possessing a degree of resistance lower than 1,000 μg/ml.
2. Speed of the lethal action of streptomycin on their sensitive cells.

Frequency of mutants with lower degrees of resistance

Our experience with routine sensitivity tests suggested that the rate of occurrence of variant cells possessing a lower degree of resistance, for example to 25 and 100 μg/ml, might offer an explanation. Accordingly, all eight species were examined to determine whether organisms capable of growth in these concentrations of streptomycin are present in all large pop-

TABLE 43

Occurrence rate of mutants resistant to 1,000 μg/ml of streptomycin

SPECIES	STRAIN 1	STRAIN 2	STRAIN 3
H. influenzae	5.7×10^{-11}	7.0×10^{-11}	2.6×10^{-11}
H. pertussis	5.2×10^{-11}	1.1×10^{-10}	1.6×10^{-10}
H. parapertussis	6.6×10^{-11}	2.0×10^{-10}	1.2×10^{-10}
E. coli	7.7×10^{-10}	2.3×10^{-10}	2.3×10^{-10}
Salmonellae	8.9×10^{-11}	3.1×10^{-10}	1.7×10^{-10}
S. typhosa	1.6×10^{-10}	1.0×10^{-10}	5.3×10^{-11}
Shigellae	3.3×10^{-10}	3.8×10^{-10}	5.3×10^{-10}
Ps. aeruginosa	4.2×10^{-10}	4.6×10^{-10}	6.0×10^{-10}

ulations and whether they exhibit the characteristics of mutants and how frequently they occur. In brief, it has been demonstrated that the organisms which on first contact with streptomycin form colonies in media containing 25 and 100 μg/ml can be found in each of these species and they also exhibit the characteristics already described for the highly resistant mutants (8). Table 44 lists for each of these species the frequency of occurrence of mutants of these lower degrees of resistance and for comparison, the incidence of those capable of growth in 1,000 μg of streptomycin per milliliter.

It is seen that the rate of occurrence of mutants of *H. influenzae* which resist the action of 25 and 100 μg of streptomycin per cubic centimeter is not different from the frequency of those which grow in 1,000 μg/cc. The same is true for *H. pertussis* and *E. coli*. The situation is quite, however, different for Salmonellae strains, *S. typhosa*, *H. parapertussis* and *Ps. aeruginosa*. Mutants of these organisms growing in 100 μg/ml occur 100 times more frequently than those resisting 1,000 μg/ml and those surviving

Experimental Basis for Predicting Therapeutic Efficacy of Streptomycin

One of the early observations, the variation in sensitivity to streptomycin of different strains of the same organism, has been assigned a position of great importance by some; this has been interpreted as an innate difference in sensitivity. *In vitro* sensitivity studies on a number of varieties of gram-negative bacilli carried out by the above procedures showed variation in sensitivity of the same strain on repeated tests; these results suggested that some chance phenomenon, operating in all strains, is responsible for the differences found in repeated tests on the same strain as well as among different strains of the same species.

The experimental studies carried out at the Babies Hospital to explore the origin of bacterial cells that resist the action of streptomycin provided the clue to the above observations. The source of the highly resistant cells (those resisting 1,000 μg/ml) was explored first. In all ten strains of *H. influenzae* studied it was possible to demonstrate the presence of cells resistant to this high concentration if sufficiently large populations were examined (3). Moreover, these highly resistant cells exhibited the characteristics of mutants and were shown to occur at a constant low rate in all ten strains (4). These results led to examination of seven other species of gram-negative bacilli, *H. pertussis*, *H. parapertussis*, *E. coli*, Shigellae, Salmonellae, *S. typhosa*, and *Ps. aeruginosa*, to determine whether their members which resist high concentrations of streptomycin are also present in all large populations and whether they exhibit traits characteristic of mutants. Mutants resistant to 1,000 μg/ml were demonstrated in each of these organisms. These experimental data provide evidence that in each of these eight species of gram-negative bacilli, emergence of resistance of the infecting organism represents a selective phenomenon (5, 6); the sensitive members are eliminated by streptomycin and the minute number of highly resistant spontaneously occurring mutants, present in any large population, make up most or all of the surviving population as a result of their reproduction.

Since the rate of occurrence of new resistant mutants of *H. influenzae* appeared to be constant for that species, it was of great interest to compare the frequencies of new highly resistant mutants of all of these species of gram-negative bacilli; the differences in therapeutic efficacy of streptomycin among these infections might be explained on this basis. The frequency of new mutants per bacterium per bacterial generation for each species is listed in table 43. These rates were calculated by using the formula of Luria and Delbruck (7). Since the method provides only an estimate, the differences in the frequencies among the species are not significant. The rate of occurrence of new highly resistant mutants is not the answer,

therefore, to the observed conspicuous differences in therapeutic efficacy of streptomycin among infections caused by these species.

In an attempt to explore the causes for these differences two factors were studied for each of the eight species:

1. The frequency of occurrence of their resistant mutants possessing a degree of resistance lower than 1,000 μg/ml.
2. Speed of the lethal action of streptomycin on their sensitive cells.

Frequency of mutants with lower degrees of resistance

Our experience with routine sensitivity tests suggested that the rate of occurrence of variant cells possessing a lower degree of resistance, for example to 25 and 100 μg/ml, might offer an explanation. Accordingly, all eight species were examined to determine whether organisms capable of growth in these concentrations of streptomycin are present in all large pop-

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H. pertussis	5.2×10^{-11}	1.1×10^{-10}	1.6×10^{-10}
H. parapertussis	6.6×10^{-11}	2.0×10^{-10}	1.2×10^{-10}
E. coli	7.7×10^{-10}	2.3×10^{-10}	2.3×10^{-10}
Salmonellae	8.9×10^{-11}	3.1×10^{-10}	1.7×10^{-10}
S. typhosa	1.6×10^{-10}	1.0×10^{-10}	5.3×10^{-11}
Shigellae	3.3×10^{-10}	3.8×10^{-10}	5.3×10^{-10}
Ps. aeruginosa	4.2×10^{-10}	4.6×10^{-10}	6.0×10^{-10}

ulations and whether they exhibit the characteristics of mutants and how frequently they occur. In brief, it has been demonstrated that the organisms which on first contact with streptomycin form colonies in media containing 25 and 100 μg/ml can be found in each of these species and they also exhibit the characteristics already described for the highly resistant mutants (8). Table 44 lists for each of these species the frequency of occurrence of mutants of these lower degrees of resistance and for comparison, the incidence of those capable of growth in 1,000 μg of streptomycin per milliliter.

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in 25 μg/ml are 10,000 to 100,000 times more frequent. In other words, mutants of *H. influenzae*, *H. pertussis*, and *E. coli* that resist the action of 25 and 100 μg/ml but are sensitive to 1,000 μg are so rare that they probably play an unimportant role in therapeutic failure of streptomycin. The much greater frequency of mutants with this degree of resistance in the other four species suggests that they play an important part in the emergence of resistance of these organisms to streptomycin and in consequent therapeutic failure of this antibiotic in the treatment of these infections.

TABLE 44

Occurrence rates of mutants resistant to 25, 100, and 1,000 μg/ml of streptomycin

SPECIES	STRAIN	25 μG	100 μG	1,000 μG
H influenzae	1	6.6×10^{-11}	5.8×10^{-11}	5.7×10^{-11}
	2	5.1×10^{-11}	3.2×10^{-11}	4.7×10^{-11}
H. pertussis	1	6.1×10^{-10}	1.1×10^{-10}	1.1×10^{-10}
	2	6.8×10^{-11}	5.2×10^{-11}	5.2×10^{-11}
E coli	1	3.8×10^{-10}	2.1×10^{-10}	2.2×10^{-10}
	2	7.2×10^{-10}	6.0×10^{-10}	7.7×10^{-10}
Salmonellae	1	9.0×10^{-7}	2.3×10^{-9}	8.9×10^{-11}
	2	4.0×10^{-6}	8.9×10^{-9}	1.7×10^{-10}
S typhosa	1	2.2×10^{-5}	1.5×10^{-6}	1.6×10^{-10}
	2	5.4×10^{-6}	2.3×10^{-8}	5.3×10^{-11}
H parapertussis	1	1.2×10^{-6}	7.0×10^{-8}	6.6×10^{-11}
	2	1.0×10^{-6}	5.2×10^{-8}	2.0×10^{-10}
Ps aeruginosa	1	7.5×10^{-6}	9.1×10^{-8}	4.6×10^{-10}
	2	1.2×10^{-5}	2.5×10^{-7}	6.0×10^{-10}

Speed of lethal action on sensitive cells

To determine rapidity of lethal action of streptomycin on the sensitive members of these species, a population of approximately 10,000 organisms was exposed to two different concentrations of streptomycin, 10 and 100 μg/ml for varying lengths of time, at intervals the number of viable organisms per cubic centimeter was determined.

Exposure to 10 μg of streptomycin per milliliter is lethal for *H. influenzae* in 2 to 6 hours and for *E. coli* in 6 hours. *H pertussis* was completely eliminated in 4 to 24 hours. *S. typhosa* and Salmonellae, on the other hand, though showing some decrease in their numbers in 6 hours,

have increased 24 hours after exposure, to a population comparable with the control. *H. parapertussis* is not reduced significantly in 6 hours, and about one-eighth of the initial populations are viable after 24 hours.

Figure 65 demonstrates that 100 μg of streptomycin per milliliter exerts a more rapid lethal action on all species. Exposure for 10 minutes is adequate to kill all *H. influenzae* organisms, 1 hour for *E. coli*, and 2 hours for *H. pertussis*. A lethal effect on 100 per cent of the other two organisms occurs at 4 hours. A small fraction of *H. parapertussis* is viable after 24 hours. It must be emphasized, however, that in clinical work a concentration as high as 1,000 μg of streptomycin per milliliter

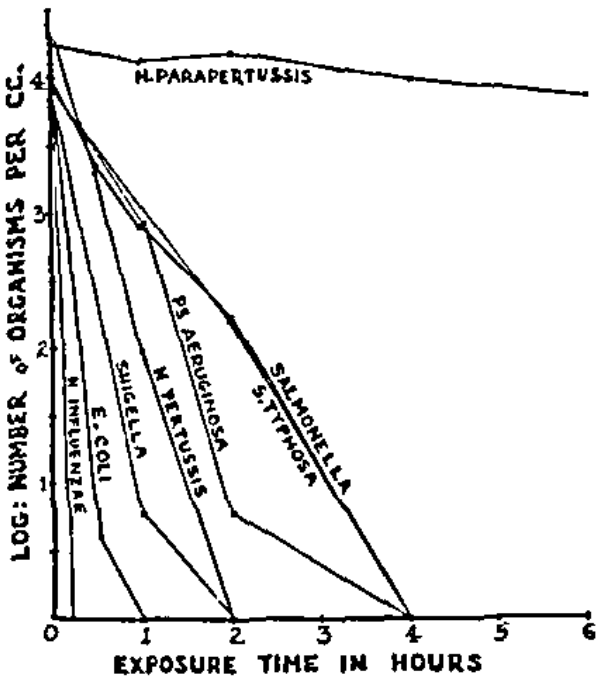

FIG. 65. Speed of lethal action of streptomycin (100 μg per ml) on different population sizes of type b *H. influenzae*.

can be obtained only in the urine and spinal fluid without serious injury to the patients from streptomycin.

The relationship of these two factors, mutation frequency and speed of lethal action to therapeutic efficacy of streptomycin in infections due to these organisms, is summarized in table 45. These data (8) suggest that the organisms causing infections which are successfully treated with streptomycin—*H. influenzae*, *E. coli*, and *H. pertussis*—have a very low incidence of mutants which resist the action of 25 and 100 μg of streptomycin per cubic centimeter; their sensitive cells are killed rapidly even by 10 μg/ml. On the other hand, in those infections in which streptomycin has not been effective therapeutically, a concentration of 10 μg/ml is not primarily bactericidal for the sensitive cells, and the frequency of mutants resistant to 25 μg/ml is high. The data along with clinical observations

in 25 μg/ml are 10,000 to 100,000 times more frequent. In other words, mutants of *H. influenzae*, *H. pertussis*, and *E. coli* that resist the action of 25 and 100 μg/ml but are sensitive to 1,000 μg are so rare that they probably play an unimportant role in therapeutic failure of streptomycin. The much greater frequency of mutants with this degree of resistance in the other four species suggests that they play an important part in the emergence of resistance of these organisms to streptomycin and in consequent therapeutic failure of this antibiotic in the treatment of these infections.

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H. influenzae	1	6.6×10^{-11}	5.8×10^{-11}	5.7×10^{-11}
	2	5.1×10^{-11}	3.2×10^{-11}	4.7×10^{-11}
H. pertussis	1	6.1×10^{-10}	1.1×10^{-10}	1.1×10^{-10}
	2	6.8×10^{-11}	5.2×10^{-11}	5.2×10^{-11}
E. coli	1	3.8×10^{-10}	2.1×10^{-10}	2.2×10^{-10}
	2	7.2×10^{-10}	6.0×10^{-10}	7.7×10^{-10}
Salmonellae	1	9.0×10^{-7}	2.3×10^{-9}	8.9×10^{-11}
	2	4.0×10^{-6}	8.9×10^{-9}	1.7×10^{-10}
S. typhosa	1	2.2×10^{-6}	1.5×10^{-8}	1.6×10^{-10}
	2	5.4×10^{-6}	2.3×10^{-8}	5.3×10^{-11}
H. parapertussis	1	1.2×10^{-6}	7.0×10^{-8}	6.6×10^{-11}
	2	1.0×10^{-6}	5.2×10^{-8}	2.0×10^{-10}
Ps. aeruginosa	1	7.5×10^{-6}	9.1×10^{-6}	4.6×10^{-10}
	2	1.2×10^{-5}	2.5×10^{-7}	6.0×10^{-10}

Speed of lethal action on sensitive cells

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H. influenzae

Except during years when meningococcus infections become epidemic, influenzal meningitis is the most frequent variety of pyogenic meningitis in infants and children. It is characteristically primary in that it develops out of a clear sky following an unimpressive upper respiratory infection; there may be coincident paranasal sinusitis or purulent otitis media, but evidence for direct extension is lacking. Bacteremia is virtually always present in untreated patients. The age incidence of meningitis was shown by Fothergill and Wright (9) to be closely related to the bactericidal power of the blood. Most infants are passively immunized *in utero*; in consequence, the incidence of this variety of meningitis is very low under 2 months of age. As this protection is lost, the disease becomes more frequent. In children aged 2 months to 3 years, the age range in which 80 per cent of the cases of influenzal meningitis occur, the blood of subjects collected at random shows only a feeble bactericidal capacity toward this organism, whereas the sera of older persons and younger infants exhibit an appreciable lethal action. The increase with age in this injurious action of blood is apparently the result of contact with the organism.

Early diagnosis is of paramount importance. Yet there are no clinical manifestations which differentiate influenzal from other varieties of meningitis. Just as in other types, the signs vary greatly from patient to patient, depending upon the age, stage of the disease, severity of the infection, and whether sulfonamides have been administered. When patients older than 7 or 8 months are seen early, the majority show only signs indicative of meningeal inflammation; the sensorium except for transient delirium is clear. In a smaller fraction the disease progresses so rapidly that the patient is comatose or semicomatose within 24 hours and the spinal fluid reveals evidence of a very severe infection; prompt recovery follows adequate treatment.

A small number of patients become comatose within 4 to 6 hours and, even though optimal therapy is applied within 12 hours of onset, they differ from others in their course. Even though the spinal fluid shows prompt improvement as evidenced by elimination of the organisms and rise in concentration of sugar, the child's clinical state persists unchanged over a period of several days and the temperature remains very high. As the sensorium clears, signs of localized cerebral damage may appear. There is reason to believe that these patients suffer from a significant degree of encephalitis secondary to damage and thrombosis of cerebral vessels. In our experience such lesions are at times compatible with complete recovery.

Another group warrants special emphasis, namely, those children who early in the course of their meningitis receive sulfonamides for an illness

suggest that knowledge of these two factors *in vitro* may serve as a reliable basis for predicting therapeutic success or failure of streptomycin in a given infection.

Clinical Aspects and Therapy of Meningitis Due to Gram-Negative Bacilli

Without specific treatment, the mortality from meningeal infection with these various gram-negative bacilli has been very high, more than 80 per cent in all age groups and close to 100 per cent in infancy, when the meningitis is a part of a generalized infection. When meningitis is caused by the introduction of gram-negative bacilli in head wounds, at operation, or by lumbar puncture for diagnosis or anesthesia, the prognosis is better.

TABLE 45

Relationship of mutation frequency and speed of lethal action to therapeutic efficacy of streptomycin

Species	Frequency of mutants resistant to concentrations of streptomycin μg per ml			Speed of lethal action per ml	
	25 μg	100 μg	1000 μg	10 μg	100 μg
H. influenzae	10^{-11}	10^{-11}	10^{-11}	2–6 hrs.	10 min.
H. pertussis	10^{-10} to $^{-11}$	10^{-10} to $^{-11}$	10^{-10} to $^{-11}$	4–24 hrs.	2 hrs.
E. coli	10^{-10}	10^{-10}	10^{-10}	6 hrs.	1 hr.
Salmonellae	10^{-6} to $^{-7}$	10^{-9}	10^{-10} to $^{-11}$	0*	2–4 hrs.
S. typhosa	10^{-5} to $^{-6}$	10^{-8}	10^{-10} to $^{-11}$	0	4–24 hrs.
H. parapertussis	10^{-6}	10^{-8}	10^{-10} to $^{-11}$	0	>24 hrs.
Ps. aeruginosa	10^{-5} to $^{-6}$	10^{-7} to $^{-8}$	10^{-10}	0	4 hrs.

* 0 = not primarily bactericidal.

Prior to the advent of streptomycin only two of these varieties, *H. influenzae* and *E. coli* group, could be successfully treated in a significant proportion of the patients. An excellent review by Paine, Murray, Sceler, and Finland (1) summarize therapeutic results prior to and since the advent of streptomycin treatment.

Although the fatality rates from meningitis caused by the various species of gram-negative bacilli do not differ significantly when these diseases are untreated, the prognosis following therapy with either sulfonamides or streptomycin does vary with the etiologic agent. The results of treatment with the available specific agents are discussed separately for each of the most frequently occurring varieties of gram-negative bacillus meningeal infections.

In the first twelve patients, treated according to our first program, streptomycin was used as the only therapeutic agent after admission to hospital unless it became evident from the poor response that amplification of this treatment was indicated. All but one patient, however, had previously received sulfadiazine. All of the patients received streptomycin alone either throughout the period of hospital treatment or for 4 days before addition of other agents. From analysis of this group it is evident that recovery was prompt in the eight patients in whom the infection was mild or moderately severe, as judged by the concentration of sugar in the spinal fluid before treatment as well as by clinical signs. On the other hand, those with severe meningitis were not cured with streptomycin alone; in two of these there was proof that emergence of resistance of the infecting organism was the cause of failure.

The failure of streptomycin alone to cure any of the four patients with severe meningitis led to the second therapeutic program. Only patients with initial spinal fluid sugar concentrations significantly above 15 mg/100 ml were to receive streptomycin alone; those with concentrations at or below this level would receive all three therapeutic agents from the start—streptomycin, sulfadiazine, and specific rabbit antiserum. This therapeutic program proved successful and was applied for the next thirteen cases.

When it was demonstrated that *H. influenzae* cells resistant to 1,000 μg/ml of streptomycin exhibit the usual degree of sensitivity to sulfadiazine (5), the third therapeutic program was instituted. Patients with meningitis of mild or average severity (spinal fluid sugar more than 15 mg/100 ml) were to be treated with streptomycin alone; those exhibiting signs of severe infection, if more than 6 months of age, received streptomycin and sulfadiazine; and in younger infants all three agents were used—sulfadiazine, streptomycin and rabbit antiserum.

The schedule of dosage of streptomycin used in the majority of patients treated at Babies Hospital is as follows: intramuscularly, an amount equivalent to 40 mg/kg of body weight is given daily, divided into eight doses; intrathecally, 25 mg is administered at a 12-hour interval for the first two doses, thereafter once daily. Treatment by all routes is discontinued after 4 or 5 days.

From the results of these therapeutic policies certain facts are clear. Sulfadiazine plays an important role as an adjunct to streptomycin. The over-all combined effect of specific rabbit antiserum and sulfonamides cannot be shown to differ significantly from the combined effect of streptomycin with sulfonamides. A higher proportion of infants less than 6 months of age have survived when streptomycin has been given in addition to serum and sulfonamides, but more of the survivors have been defective. Table 46 summarizes the results of streptomycin therapy alone and with adjuncts. In choosing between rabbit antiserum and sulfadiazine, on the

which is not recognized as meningitis. Doubtless, some of the mildest recover completely, but in others the signs of meningeal inflammation are completely masked, fever persisting as the sole clinical evidence of disease Withdrawal of the sulfonamides during an observation period is usually followed by the appearance of clear signs of meningeal irritation.

In 1938 and during the next several years a therapeutic program for treatment of *H. influenzae* meningitis which proved highly successful (10, 11) was developed at the Babies Hospital. There is reason to believe that when the combined action of sulfadiazine and specific rabbit antibody is applied according to certain principles early in the disease it is possible to cure 100 per cent of the patients. The response is so consistent that one can predict not only the outcome but the course of recovery. Even in the fulminating group in which the meningitis progresses so rapidly that the spinal fluid sugar falls to less than 15 mg/100 ml within 24 hours of onset, prompt recovery can be expected in all cases if sufficient antibody is administered in the initial dose. Actually only 80 per cent of the ninety patients treated according to this regimen recovered, the failures being attributable to delay in diagnosis and to unwarranted confidence in the value of sulfonamides alone.

After demonstration of the rapid lethal injury resulting from the action of streptomycin on type b *H. influenzae*, *in vitro* and in the mouse, the therapeutic value of the drug in influenzal meningitis was explored. Investigation of the separate action of streptomycin in influenzal meningitis has been difficult for two reasons: first, because most of the patients had already received sulfonamides [illegible] lation; and second, because there had been shown to be capable of curing virtually 100 per cent of [illegible] when applied according to certain principles in the first few days of the disease. Nevertheless, this trial was considered necessary because, if found equally effective, streptomycin could be expected to possess certain advantages over the earlier therapeutic program; serum sickness could be eliminated. Streptomycin has been shown to be active against all six types of encapsulated *H. influenzae* as well as the nonencapsulated, nontypable variety of this organism which occasionally causes subacute bacterial endocarditis in children and adults and meningitis in very young infants. This is true of sulfadiazine also, but type b *H. influenzae* antiserum, the only therapeutic serum available at present, is effective only against type b. More than 95 per cent of all serious infections caused by the Hemophilus group are due, however, to type b

During the last 3 years four different therapeutic programs have been used in an effort to evaluate streptomycin in influenzal meningitis. The changes have resulted from clinical experience as well as from experimental results on the action of streptomycin on type b *H. influenzae* (12).

mately 100 μg/ml, was studied to determine its *in vitro* effect on different population sizes of *H. influenzae* after exposure for different lengths of time. Figure 66 shows these results when experiments were carried out in broth. An exposure for 10 minutes killed 100 per cent of the small population, 5,000 to 10,000 organisms The 130 million population required 2 hours for complete elimination, and the 1.3 billion approximately 6 hours.

TABLE 47

Concentration of streptomycin in spinal fluids of patients receiving 25 mg intrathecally

TIME AFTER ADMINISTRATION	PATIENT I STREPTOMYCIN	PATIENT II STREPTOMYCIN
	μg/cc	μg/cc
½ hr.	410	
1 hr.	368	204
2 hrs.	74	149
3 hrs.	104	
24 hrs	6	7

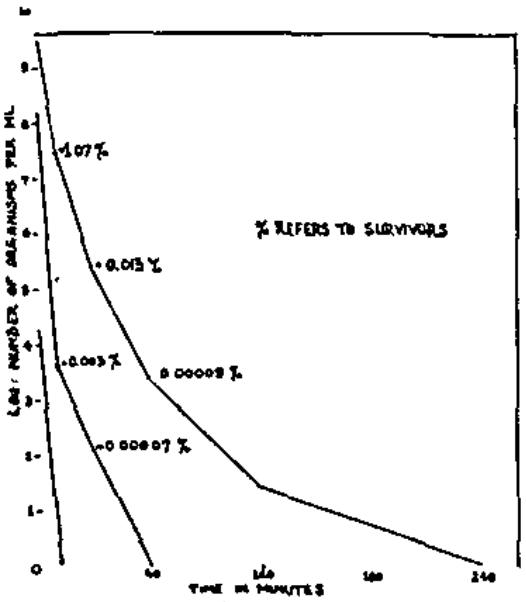

FIG. 66

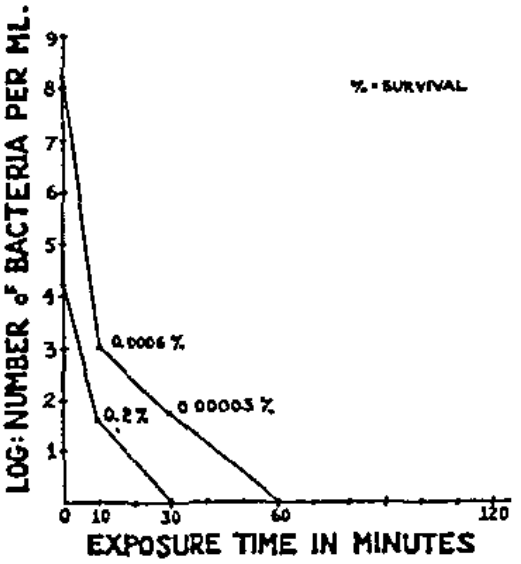

FIG. 67

FIG. 66. Rapidity of lethal action of 100 μg per ml of streptomycin in broth.
FIG. 67. Speed of lethal action of 100 μg per ml streptomycin in spinal fluid.

Figure 67 shows comparable results of similar experiments carried out in spinal fluid.

To determine the range of size of the bacterial populations encountered in influenzal meningitis prior to treatment, spinal fluids from nineteen patients have been studied. The figures in table 48 present the number of viable organisms per ml of spinal fluid and must therefore be multiplied by approximately 100 to obtain the total population. It is seen that the

one hand, and streptomycin and sulfadiazine, on the other, since they appear to be equally effective, one must weigh the expense of the antiserum against the toxicity of streptomycin. Damage to the vestibular apparatus has been the most frequent sequela of streptomycin therapy administered for long periods; acoustic nerve deafness is unusual unless very high concentrations are allowed to accumulate in the blood following large doses or failure of excretion because of kidney damage. A small proportion of

TABLE 46

Summary of therapeutic results of treatment with streptomycin alone and with adjuncts

SEVERITY	THERAPY	NUMBER OF PATIENTS	SUCCESS	FAILURE	AFTER ADDITIONAL THERAPY		
					R	S	D
Mild to average	SM alone throughout	22	22	0			
Severe	SM alone	8	0	8	4	2	2
Average	SM + SD + serum	4	4				
Severe	SM + SD + serum	7	6	1			
Average	SM + SD	1	1	0			
Severe	SM + SD	7	7	0			
Mild to severe	SM only 24 hours + SD	4	4	0			

R = Recoveries SM = Streptomycin
S = Survivals SD = Sulfadiazine
D = Deaths

Streptomycin alone for at least 4 days
30 patients—73 per cent successful (all in mild or average group)
Total number patients 53
46 patients—88 per cent apparently complete recovery
4 patients—survived but defective mentally and physically
3 patients—fatal

children receiving streptomycin for only 4 or 5 days in the dosage outlined exhibit complete elimination of vestibular function.

For these reasons, investigations have been carried out to learn whether the period of streptomycin therapy can be shortened sufficiently to eliminate vestibular damage (13). *In vitro* experiments explored the shortest period of exposure to streptomycin that is effective in eliminating all of the organisms. To determine what concentration of streptomycin should be used in these experiments, concentrations of the drug in spinal fluid of two patients were measured after intrathecal introduction of 25 mg; the results are shown in table 47. The concentration remaining after 3 hours, approxi-

mg will be adequate for the specific chemotherapeutic effect of this antibiotic and, when used along with sulfadiazine, can be expected to cure the majority of patients. These principles have been applied to the treatment of four patients; the results are summarized in table 49. Recovery was rapid and complete; the cultures became sterile within 18 hours and the temperatures normal in 24 to 72 hours, even though two patients exhibited signs of severe infection.

Colon bacillus

E. coli is responsible for approximately 30 per cent of the cases of meningitis in infants during the first 4 to 6 weeks of life. The various theories

TABLE 49

Patients treated with streptomycin and sulfadiazine for 24 hours

PATIENT	AGE	DURATION MENINGITIS	PREVIOUS THERAPY	SPINAL FLUID			TEMPERATURE NORMAL
				Sugar	Bacteria	Sterile	
	months	*days*	*days*	*mg/100*	*per ml*	*hours*	*hours*
KS	17	5	SD2 P1	13	22,000,000	12	24
GK	15	1	SD½ P½	71	21	12	24
TG	20	1	SD1 P1	17	· —	12	24
BG	36	2	P1	14	11,800,000	18	72

SD = Sulfadiazine. P = Penicillin.

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These experimental results led to our fourth therapeutic program, which is under investigation at present. The following treatment is administered to all patients in the early stage of influenzal meningitis: Streptomycin is administered for only 24 hours; it is given intramuscularly in an amount equivalent to 40 mg/kg in eight doses, and intrathecally in one dose of 25 mg. Sodium sulfadiazine (0.1 gm/kg) is given subcutaneously at 12-hour

TABLE 48

Correlation between concentration of sugar and size of bacterial population in nineteen patients

PATIENT	SPINAL FLUID SUGAR CONCENTRATION BEFORE TREATMENT	VIABLE ORGANISMS PER MILLILITER OF INITIAL SPINAL FLUID
	mg/100	
1	5	334,000,000
2	9	356,000,000
3	6	85,000,000
4	13	48,000,000
5	11	24,000,000
6	14	11,800,000
7	27	4,000,000
8	33	1,820,000
9	18	72,000
10	24	17,000
11	29	8,340
12	24	6,000
13	38	4,600
14	38	2,140
15	44	1,830
16	23	660
17	28	44
18	74	24
19	29	20

intervals for at least 24 hours; thereafter it is given for 6 additional days orally as soon as the patient can drink without vomiting. For two reasons this treatment is limited to patients in the early stage: the circulation of the spinal fluid is still free, permitting exposure of all areas of the meninges to the high concentrations of streptomycin which follow intrathecal injection, and the likelihood of resistance to sulfonamides is remote. When the latter drugs have been used ineffectively for some time, the enhancement of streptomycin therapy by these agents may be lost because of emergence of resistance. A limited clinical trial suggests that a 24-hour period of intramuscular streptomycin administration and one intrathecal dose of 25

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TABLE 50

Therapeutic results with streptomycin in meningitis due to gram-negative bacilli other than H. influenzae

SPECIES	INVESTIGATOR	TOTAL CASES	RESULTS OF TREATMENT						OVER-ALL MORTALITY	NO GROSS DEFECTS
			SM alone			SM + SD				
			R	S	D	R	S	D		
									per cent	*per cent*
E. coli	Keefer-Hewitt (16)	12				6	3	3		
	Paine *et al.* (1)	7	5			1		1		
	Amos Christie*	1						1		
	Edward C. Curnen*	2					2			
	Wilburt C. Davison*	1					1			
	Robert Ward*	1						1		
	Total	24	5			7	6	6	25	50
Ps. aeruginosa	Keefer-Hewitt (16)	11				6		5		
	Paine *et al.* (1)	8	2		1	2		3		
	Stanley, M. M. (17)	1						1		
	Weinstein *et al.* (21)	3				3				
	Total	23	2		1	11		9	43	57
Kl. pneumoniae	Fisher, G. J. (25)	1				1				
	Keefer-Hewitt (16)	4				2		2		
	Paine *et al.* (1)	5						5		
	Maxwell Finland*	1			1					
	Tartakoff *et al.* (24)	1						1		
	Total	12			1	3		8	75	25
Proteus	Keefer-Hewitt (16)	3	2		1					
	Maxwell Finland*	1			1					
	Lepow, H. *et al.* (29)	1						1		
	Paine *et al.* (1)	2	1		1					
	Total	7	3		3			1		
Salmonellae	Paine *et al.* (1)	4	1		1		1	1		
	Horace Gezon*	1					1			
	Total	5	1		1		2	1		
A. aerogenes	Keefer-Hewitt (16)	1						1		
	Paine *et al.* (1)	2	1		1					
	Total	3	1		1			1		
A. faecalis	Keefer-Hewitt (16)	3	1		2					
	Paine *et al.* (1)	3	1		2					
	Terry, L. L. *et al.* (31)	1				1				
	Total	7	2		4	1				

SM = Streptomycin. SD = Sulfadiazine.
R = Recoveries. S = Survivals. D = Deaths.
* Personal communication.

congenital dermal ninus extends to the meninges. Recognition of such a source is important, since surgical excision of the sinus may be necessary to prevent recurrence. Mount has reviewed the literature and reported 5 additional patients (18).

The previous high mortality from this variety of meningitis has been decreased by sulfonamide treatment; however, these drugs fail in a significant number of patients.

Since a large proportion of patients with colon bacillus meningitis are young infants, the infections are likely to be severe and firmly established before treatment is instituted. Even though experimental work shows that streptomycin has a rapid bactericidal effect on sensitive *E. coli* organisms, large populations will inevitably include resistant cells, and therefore a sulfonamide is an important part of treatment initially. The results of the limited experience with streptomycin therapy are summarized table 50.

Ps. acruginosa

Meningitis due to *Ps. aeruginosa* is usually a complication of serious debilitating disease or results from introduction of the organism as a contaminant from a head wound or from lumbar puncture for a diagnosis or spinal anesthesia. The mortality is quite different in the two groups: in the former, without specific therapy, more than 85 per cent are fatal; and in the latter, about 55 per cent. Pyocyaneus meningitis following introduction of intrathecal penicillin from contaminated syringes has been reported by Harris, Buxbaum, and Appelbaum (19). The bacteremia which at times follows instrumentation of the urinary tract may be the source. Experience prior to 1947 has been reviewed by Paine, Murray, Harris, and Finland (20). They have collected from the literature twenty-one cases treated with sulfonamides and penicillin; fifteen were fatal. Weinstein and Perrin (21) have reviewed eighty-two cases and added three of their own which followed spinal anesthesia. Only a small number of patients have been treated with streptomycin. These are listed in table 50.

Kl. pneumoniae (*Friedländer's bacillus*) *meningitis*

This variety of meningitis has been reported very infrequently. In 1943 Ransmeier and Major (22) collected twenty-nine cases from previous publications and reported another. Analysis of this group shows that in about half of the cases the meningitis followed infections of the middle ear or paranasal sinuses. In the others, it was associated with pneumonia, cholecystitis, or colitis with bacteremia. Solomon (23) reported three patients in whom a fatal meningitis was associated with infected wounds; sulfadiazine and penicillin used in two of these were unsuccessful. Tartakoff, Grynbaum, and Le Compte (24) reported a case of Friedländer's bacillus meningitis following operation for a meningioma. The spinal fluid

Proteus meningitis

Twenty-one cases of Proteus meningitis have been reviewed by Lepow, Friedenthal, and Jaffe (29). Of these, thirteen were secondary to otitis media, and a focus could not be found in seven. Bacteremia was demonstrated in four. Of the fifteen cases reported prior to the use of sulfonamides, 86.7 per cent were fatal. Of the seven patients treated with sulfonamides, three recovered and four died. The authors reported a fatal outcome in one patient of their own, treated with streptomycin, penicillin, and sulfadiazine, apparently rather late in the course of the disease. The reports on results of streptomycin therapy are listed in table 50.

P. tularensis meningitis

Five cases of tularemic meningitis have been reported (30); all occurred prior to the advent of streptomycin and were fatal. From the outstanding success of streptomycin therapy in systemic tularemia there is every reason to believe that meningitis will also respond if treated early.

Other varieties of gram-negative bacillus meningitis

A great variety of other gram-negative bacilli may cause meningitis but even less frequently than those just described. Any organism that normally inhabits the intestinal tract may infect the meninges following bacteremia or contamination of the spinal fluid through head injuries, congenital defects (for example, meningocele), or lumbar puncture. Terry, McBane and Dean (31) have reviewed four cases of meningitis caused by *A. faecalis* and reported one of their own treated successfully with streptomycin and sulfadiazine. The other varieties that have been treated with streptomycin are listed in table 50 As emphasized by Paine, Murray, Seeler, and Finland (1), approximately half of these patients died but the late stage of the disease played an important role.

From the review of this experience it is clear that certain points deserve emphasis. The number of reports of development of meningitis from contamination of spinal fluid during diagnostic and therapeutic procedures and spinal anesthesia points out the need for stricter aseptic technique. The need for accurate bacteriologic diagnosis is also apparent; the therapeutic requirements differ with the species When gram-negative bacilli are seen on stained smear the commonest variety, *H. influenzae*, can be identified within 30 minutes by demonstration of capsular swelling with type-specific diagnostic serum. When this reaction is absent, the spinal fluid should be seeded on an enteric medium, as for example McConkey's or SS agar (Difco), in addition to Levinthal and blood agar; this saves time

became sterile after treatment with sulfadiazine and penicillin for 4 days. Subsequent administration of streptomycin, 500 mg intrathecally and 1 gm every 8 hours intravenously, was followed by death of the patient; at postmortem the meningitis had cleared. Paine, Murray, Seeler, and Finland (1) summarize the case reports of four previously reported patients who were treated with streptomycin for *Kl. pneumoniae* meningitis; all of these patients died. Fisher (25) has brought the review of past experience up to date and reported successful outcome in one patient after sulfadiazine, streptomycin, and penicillin therapy.

Although the sulfonamides have been used successfully in some patients even in infancy (26), their action is clearly limited. Streptomycin has been used in too small a group for evaluation. The marked *in vitro* and *in vivo* sensitivity of *Kl. pneumoniae* to streptomycin suggest, however, that this antibiotic is the most effective single agent. On the other hand, streptomycin can be expected to be limited because of emergence of resistance in severe infections and also because of the frequency of serious underlying disease. Therefore, both sulfonamides and streptomycin are indicated initially in all patients with this variety of meningitis. The results of treatment with streptomycin in a few cases listed in table 50 were very poor, but the serious underlying disease doubtless played an important role.

Salmonella meningitis

A review of this subject by Neter (27) summarizes past experience. This variety of meningitis is associated with a bacteremia secondary to Salmonella enteritis, it is rare except in seriously ill infants. Under the latter circumstances it is often unrecognized until postmortem examination. Recoveries have been reported after various sulfonamide therapies (28). Yet the limitations of sulfonamides in Salmonella infection outside the central nervous system are well known.

Systemic Salmonella infections have not been successfully treated with streptomycin. In the five reported cases listed in table 50 the spinal fluid was sterilized in three but in two of these serious cerebral damage persisted. The experimental data suggest that the high concentrations of streptomycin that persist in the spinal fluid after an intrathecal dose of 25 mg will be adequate to eliminate relatively small bacterial populations.

which acts through a different mechanism. The action of sulfonamides in this role can be expected to be limited Polymyxin B, on the other hand, exerts a powerful bactericidal action against both streptomycin-resistant and -sensitive cells.

the survival of defective children. When *H. influenzae* is excluded, the majority of the patients who develop gram-negative bacillus meningitis are under 6 months of age. In a significant fraction of these patients, infection of the meninges is not discovered until postmortem examination. These infants fail to develop the usual signs that lead to the suspicion of meningitis until the advanced stage is reached. Different criteria must be used as indications for performing lumbar puncture to diagnose meningitis in its early stage: increased tension of the fontanel, a staring expression, drowsiness alternating with irritability, a high-pitched cry, or persistent unexplained fever.

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in differentiation of a member of the *Hemophilus* group from the enteric species.

Conclusions on streptomycin therapy

Streptomycin is the most important single agent which has had clinical trial in meningitis due to gram-negative bacilli. Until there is proof that intrathecal streptomycin is unnecessary, administration by this route is indicated. There is reason to believe that one accomplishes little by increasing the intramuscular dose above 40 mg/kg body weight daily in eight doses and the intrathecal dose beyond 25 to 50 mg at each injection; 25 mg is recommended for patients under 5 years. The elimination of vestibular function in a small fraction of patients treated for only 5 days according to this dosage demands that the duration of streptomycin therapy be reduced to a minimum. In *H. influenzae* meningitis a 24-hour period of intramuscular injections and one intrathecal dose appear to be enough. In some varieties, for example pyocyaneus, Salmonella, or typhoid meningitis, 25- to 50-mg doses given intrathecally at 12-hour intervals for four doses and continuation of intramuscular treatment for 48 hours may be indicated. There is reason to believe that intrathecal streptomycin is not injurious when administered according to this principle. The experimental data suggest that the maximum streptomycin action occurs over a short period, not exceeding 48 hours after exposure of the organisms; those which persist are probably resistant and will require an agent working through a different mechanism. Of those which have had clinical trial, sulfadiazine is the adjunct of choice in meningitis due to *H. influenzae*, *E. coli*, or *Kl. pneumoniae*. It was pointed out previously that mutants of *H. influenzae*, *E. coli*, and Shigellae which are highly resistant to streptomycin have been shown to exhibit normal sensitivity to sulfadiazine. In

of polymyxin B is much greater. Although the latter antibiotic has not yet been proved to be free of a renal irritation factor, it has been used intrathecally without untoward effects (32). One or two intrathecal injections can be expected to produce its maximal specific action, since its bactericidal action on some organisms, at least, is even more rapid than that of streptomycin. The concentration of sugar in the spinal fluid before treatment offers a valuable guide to the number of adjuncts needed.

Regardless of the high degree of efficiency that may be attained by use of the combined action of agents operating through different mechanisms, in eliminating all organisms from the spinal fluid, unless they are applied before irreparable damage has been done such treatment will only permit

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Chapter 23

TULAREMIA

Streptomycin is already by unanimous agreement the most effective available agent for the treatment of tularemia (1). This is conspicuously true for the natural disease in man, and for the experimental disease in animals, including the white mouse, which is well known to be totally nonresistant to challenge with one viable cell of a virulent strain. Since man-to-man transmission is so rare that it may be said practically not to occur, there arises no anxiety concerning possible social consequences of the development of microbial resistance. Streptomycin is so highly effective that so far as recovery or the amelioration of severe symptoms is concerned most patients require no additional therapy

The aims of therapy are to prevent deaths at the usual critical stage near the beginning of the third week of disease; to prevent all possible deaths from fulminating infections which kill in 4 to 10 days; to ameliorate the severe and disabling symptoms; to prevent many bronchopulmonary lesions from becoming clinically manifest, and to keep manifest pneumonias and serosal lesions from growing large enough to cause analogues of the Herxheimer reaction when they are treated; to diminish the risk of suppurative adenitis; to prevent ulceration and the consequent risk of secondary infection of primary lesions; to administer enough streptomycin for a long enough period to prevent therapeutic relapse, and yet to avoid excessive dosage so as to incur the fewest possible instances of toxic reactions

Clinical Study

The composition of a group of 91 patients who were treated with streptomycin and, for comparison, the frequencies of clinical types and of pneumonia by type from a large unselected group are shown in table 51. The highest type fatality rate, about 24 per cent, is caused by the typhoidal type, and the supervention of pneumonia normally predicates a higher mortality from all clinical types. The treated group was highly selected for the typhoidal type and for pneumonia in all types, and the figures for morbidity and mortality shown in table 52 are highly significant. This group comprises many of the earliest patients to receive streptomycin, and

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onset of therapy ranging from the first to the 123rd days of disease. Two deaths occurred: one consequent upon lung abscess formation, the other on the sixth day of disease shortly after treatment was initiated.

The individual effects of therapy were equally striking. During the first to third days of administration there occurred marked relief from headache, depression, and severe malaise; lowering of fever to normal; dwindling diameters of buboes; and lessening of pain in buboes and in primary lesions. Enlarging pulmonary exudates were usually but not always checked during the first few days. Stupor was often dispelled by the second or third day. Many patients survived who at the onset of therapy were semicomatose or comatose, and who had unremitting high fever, a combination marking the worst possible prognosis.

The rate for suppurative adenitis was barely halved, and this relatively minor but distressing sequel caused significant prolongation of fever, disease, and disability. The larger the bubo at onset of therapy, the greater the likelihood of suppuration, and resolution occurred in but few that reached or exceeded a diameter of 5 cm. A rough analysis of the meager data showed no correlation between total streptomycin dosage and suppuration but did suggest some relationship to the length of the administration period; administration for 6 or more days showing less suppuration than from any dosage administered for less than 5 days.

Drug fever occurred in three patients who received total dosages of 13.5, 7.8, and 9.3 gm at daily rates of 3, 1, and 1.2 gm, respectively. Transitory tinnitus and circumoral numbness followed the administration of 21 gm during 7 days. Vertigo, with or without tinnitus, occurred in four patients who received 9 3, 5, 42, and 15 gm at daily rates of 1.2, 1, 3, and 1 gm, respectively Transitory headache, nausea, muscle cramps, or general mild malaise occurred in six patients who received 5 gm or less at rates of 1 gm or less daily. Vertigo persisted in moderate degree for 5 or more months in a woman who received 9 3 gm at a rate of 1.2 gm daily. Through the 25th month after cessation of therapy she was still having difficulty in going down stairs, getting off cars, stepping off curbs; a firm grip on something stable was required to prevent falling.

Five patients in the series experienced analogues of the Herxheimer reaction, and the literature already contains at least as many. The clinical chart for Cohen and Lasser's case is illustrative (2).

Discussion

Although all major aims of therapy are obviously being achieved, the degree of success with some of the minor ones is less conspicuous. Intramuscular administration remains the method of choice, but a continued insistence upon frequent injections, 3 or 4 hours apart, is not only un-

the figures include the resultant effects of errors of judgment and procedure. Although therapy was started as late as the average 20th day of disease, the mortality was reduced to one-third, and the major aspects of morbidity were reduced to about one-half those of the control group. The convales-

TABLE 51

Frequency of clinical types of tularemia and of pneumonia in a streptomycin-treated group compared with unselected type and pneumonia frequencies

	STREPTOMYCIN-TREATED N = 91				UNSELECTED N = 832	
	Clinical type no	Type frequency	Number with pneumonia	Type pneumonia frequency	Type frequency	Type pneumonia frequency
		per cent		*per cent*	*per cent*	*per cent*
Ulceroglandular . . .	65	71.4	18	28	87	15
Glandular	2	2.2	1	50	3	13
Oculoglandular .	2	2 2	1	50	2	10
Typhoidal .	22	24.2	20	91	8	51
Totals . .	91		40	44		18

TABLE 52

Comparison of means from control group with averages for streptomycin-treated group

		UNTREATED N = 542	TREATED WITH STREPTOMYCIN N = 91
Duration of			
Disease	*months*	3 78	*1.90*
Disability	*months*	3 12	1 92
Adenopathy	*months*	3 50	1.56
Fever	*days*	30.6	26.1
Bedridden	*days*	46 8	23.7
Primary lesions	*days*	40 6	24 5
Therapy-to-recovery interval	*days*		39
Day of disease therapy started			19.7
Suppurative adenitis, per cent		56	29
Mortality, per cent		6	2 2

cent period, measured from the day therapy was started to the day uninterrupted full-time work could be resumed, averaged 39 days These significant changes in the course of disease were accomplished by widely varying total dosages, from 0.64 to 42 gm, an average of 7.86 gm per patient, usually administered intramuscularly every 3 or 4 hours, and with

may safely assume that any dosage, maintained for 5 days, that was adequate to induce vast clinical improvement would be extremely unlikely to permit acute relapse, and that if the initial dosage caused in addition a possible Herxheimer reaction, then the provisional resumption of this same dosage would be an adequate guard against the feared alternative of therapeutic relapse. *And under these conditions and time relations there could* be no question of acquisition of microbial resistance to the antibiotic.

The detailed pathogenesis of suppurative lymphadenitis is not clearly understood, and this may in part account for the relatively poor showing of otherwise adequate treatment in the prevention of liquefaction of buboes. There is no satisfactory explanation for the subsequent enlargement and suppuration of nodes that were invisible and impalpable during the well-treated acute phase or of those which receded promptly to impalpable status under treatment. Yet suppuration under these circumstances has occurred as early as 10 days after cessation of treatment, and more frequently during the ensuing 8 weeks. Since these events have also occurred in patients treated with serum therapy, and in many who received no special form of treatment, even with abrupt reenlargement and rapid liquefaction as late as the 34th month after apparent termination of the disease, it may be inferred that some bacteria become lodged within certain cells of a node, *with or without benefit of therapy, and that the established equilibrium* is subject to perturbations that may at times cause a reactivated local lesion. *The associated minor to trivial constitutional symptoms are consistent* with an endogenous local reinfection in an otherwise solidly immune person, as Francis first noted in relation to exogenous local reinfections (5). This hypothesis implies a defective defense apparatus rather than a failure of the antibiotic, which may merely have been administered at a time when some bacteria were shielded from its action.

Unless it is possible to effect a total kill of *all* bacteria within each patient by streptomycin, it may not be even theoretically possible to prevent all suppurative adenopathy, an idea not inconsistent with all recorded experience to date. Since serum agglutinins are formed and maintained for years despite massive therapy during the earliest stages of the disease, implying continuous new antibody formation and probably the continued presence of living antigen, there is yet no evidence that streptomycin has killed every infecting bacterium in any patient Nor has such evidence yet been obtained for the recovered experimentally infected animal. In the interest of lowest frequency of suppurative adenitis, however, it is justifiable to administer streptomycin as early as possible, before buboes become large, to give it for at least 6 days if buboes are present, and to increase both the administration period and the daily dose if buboes are larger than 3 cm. It is apparent that much more streptomycin is needed

necessary for most patients with this disease but is an unjustifiable source of discomfort and broken sleep. Calloway's (3) experience that the patient with tularemia of average severity does extremely well with injections every 8 hours is being confirmed by others and is a step in the right direction. The possibility of satisfactory response to injections every 12 hours should be explored in suitably selected patients.

Many early case reports illustrated a tendency toward excessive dosage, some apparently owing to a failure to appreciate the efficacy of the antibiotic in this disease, and some obviously due to misinterpretation of the Herxheimer type of reaction. Since prolonged vestibular dysfunction has followed the administration of 1.2 gm daily for 8 days, and since there is no available evidence to justify the use of more than 6 gm per patient, the trend toward smaller dosage, well exemplified by Hunt's excellent study (4) in which 3.5 gm was the average total dose for the case of average severity, is sound and consistent with accumulating experience. It seems probable that a total dose of 2 to 3 gm per patient, as 0.5 gm a day for 2 days followed by 0.25 gm a day for 4 days, or else as 0.5 gm daily for 6 days, all injections being given 8 hours apart, will suffice for most cases. The psychic state and the nature of the temperature curve will probably continue to be the best clinical guide to dosage.

Tularemic exudates contain numerous bacteria and macrophage cells. Phagocytosis and solubilization of bacteria become enormously accelerated when the local concentration of streptomycin reaches a bacteriostatic level. Soluble bacterial products then diffuse into the blood, where they characteristically cause a lowering of previously determined agglutinin titers or, less easy to verify, a maintenance of a low titer far beyond the time at which the titer normally would show a marked rise, also, if the source exudates are large they may provoke an exacerbation of symptoms characteristic of tularemia and a recurrence of fever to moderate degree. The fever, with or without malaise, headache, and drowsiness, may persist for 10 days or longer, usually terminating at the time the first evidence of resolution of the exudate is obtained, although its decline is often attributed to additional, often intensive, streptomycin therapy, the removal of pleural fluid, or other coincident event The Herxheimer type of reaction should be anticipated whenever *large* pulmonic or serosal exudates are present. The *in vitro* agglutinin-absorption phenomena are reliable differential diagnostic criteria if agglutinins are present, in their absence, discrimination between this reaction and a relapse of the disease is possible by careful examination of the patient, who, despite the fever and even despite a considerable degree of malaise and mental dulness, usually can be shown to be actually better day by day even though the fever is still rising. In tularemia, unless further experience should indicate to the contrary, one

may safely assume that any dosage, maintained for 5 days, that was adequate to induce vast clinical improvement would be extremely unlikely to permit acute relapse, and that if the initial dosage caused in addition a possible Herxheimer reaction, then the provisional resumption of this same dosage would be an adequate guard against the feared alternative of therapeutic relapse. And under these conditions and time relations there could be no question of acquisition of microbial resistance to the antibiotic.

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to prevent some buboes from suppurating than is necessary to prevent deaths from extensive tularemic pneumonias. However, the prevention of suppuration of a bubo at the risk of even a short-term loss of hearing or of equilibrium seems an excessive price for the gain. Perhaps the use of dihydrostreptomycin will provide a practical solution.

The relapsing patient who was not treated during the acute phase of disease, and who obtained only transitory or no improvement from ample treatment (5 to 6 gm) at a later stage (6), seems to represent another expression of the same fundamental host: parasite relationship that permits recurrent lymphadenitis. The nature of the symptoms and the concomitant *in vivo* agglutinin absorptions indicate that the relapses are endogenous reinfections in partly immunized persons, that the bacteriostatic equilibrium of each relapsing person was demonstrably unstable before treatment was first given. Since two other patients of this series obtained dramatically prompt recoveries from streptomycin first administered during the 4th and 5th months of disease, respectively, there is no support for the opinion that mere chronicity of infection prior to therapy is a cause for therapeutic failure.

Regional localizations respond well; conjunctivitis, pneumonia, pleurisy, *pericarditis, peritonitis, splenitis, hepatitis, and meningitis* or meningoencephalitis have been treated successfully. Recovery from meningitis has been induced with and without intrathecal therapy. Experience is too limited to appraise the need for or the risks of intrathecal therapy, but at present this seems to be unnecessary.

Treatment should be instituted as early as possible to achieve best the stated aims. There is yet no indication that continued disease of any duration would not respond well. The problem of the fulminant case is one solely of early diagnosis and early therapy, the former made by necessity entirely on clinical and epidemiologic grounds without loss of precious time in futile efforts to obtain laboratory confirmation. Verification is always possible by subsequent agglutination tests if the patient survives. There is no evidence that the earliest treatment interferes with the development of the usual solid immunity after recovery.

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CHAPTER 24

BRUCELLOSIS

Since the discovery of the etiology of brucellosis by Bruce over a half century ago, many agents have been evaluated in the therapy of this disease. From time to time startling therapeutic claims have been made for this or that drug, but critical clinical evaluation has eventually eliminated each one of them. Introduction of the sulfonamides into the therapy of infectious diseases aroused high hopes that at last specific treatment would be available for brucellosis. Occasional cases of acute brucellosis have undoubtedly responded favorably to the use of such drugs as sulfanilamide and sulfadiazine. In general, however, sulfonamide therapy did not completely control the disease, and relapses occurred frequently. It was readily demonstrated that penicillin was without effect either in experimentally infected animals or in human cases. It is understood, therefore, why considerable attention was given to the evaluation of streptomycin in human brucellosis. Early investigations indicated this new antibiotic was markedly inhibitory for gram-negative bacilli

NATURAL HISTORY OF BRUCELLOSIS

Before the present status of streptomycin in the treatment of human brucellosis is set forth, some of the essential features of the clinical course of this disease should be recalled. It is important to bear in mind that human brucellosis may be caused by one of three species of Brucella; namely *Br. melitensis*, *Br. suis*, and *Br. abortus*. The most severe infections are caused by *Br. melitensis*, and the mildest, by *Br. abortus*, though there are many exceptions to this general statement. Brucellosis may exist as an acute febrile disease which may terminate spontaneously after a few days or weeks of illness; or the illness may have an insidious onset, and active disease may persist for months or years. It is unfortunate that brucellosis has been diagnosed only too frequently on the basis of inadequate data. In strict clinical investigation, only bacteriologically proved cases of brucellosis can form the basis for evaluating any specific agent.

Streptomycin in Experimental Brucellosis

Early investigations in the laboratory looked very promising, and it appeared that at long last streptomycin was the specific agent for brucellosis. Jones, Metzger, Schatz, and Waksman (1) showed that infected chick embryos could be protected by streptomycin. Conflicting results with streptomycin in experimentally infected guinea pigs were obtained, Live, Sperling, and Stubbs (2) maintaining that the animals were protected, but Kelly and Henley (3) could not confirm this. Hall and Spink (4) tested the *in vitro* sensitivity to streptomycin of a large number of strains freshly isolated from human beings and observed that growth of all the strains was inhibited by concentrations of the drug which could readily be attained in human beings. The observations of Jones *et al.* were confirmed in that streptomycin protected experimentally infected chick embryos (5, 6).

Streptomycin in Human Brucellosis

Although it had been demonstrated *in vitro* that Brucella cells were sensitive to the action of streptomycin, a paradoxical biological phenomenon was soon encountered when the drug was administered to human cases of brucellosis. Several groups recorded disappointing clinical results (7, 8, 9, 10, 11) In a few instances, more encouraging results were obtained (12). But with the passage of time, and as more cases due to each of the three species of Brucella were treated with streptomycin, it became apparent that this antibiotic was not the answer to the therapy of brucellosis.

It is not clear why streptomycin should not favorably alter the clinical course of human brucellosis. Clinical failures have not been due to the appearance of streptomycin-resistant strains of Brucella. It is possible that the type of tissue reactions induced by Brucella may be related to the failure of streptomycin to control the disease Intracellular parasitism is a characteristic feature of brucellosis, and it may be that Brucella organisms are protected against the antibiotic within the confines of the tissue cells (13). In favor of this hypothesis is the observation that streptomycin may cause temporary improvement in a seriously ill patient with brucellosis, but a demonstrable bacteremia may appear shortly after therapy is discontinued, an occurrence often associated with a clinical relapse

Combined Streptomycin-Sulfadiazine Therapy in Human Brucellosis

Pulaski and Amspacher (9) reported that streptomycin was without benefit in seventeen patients having acute or chronic brucellosis. But when sulfadiazine was given simultaneously with streptomycin to two patients with acute brucellosis, there was a prompt response. On the basis of this latter observation, these investigators recommended that for the

treatment of brucellosis 0.5 gm of streptomycin should be given intramuscularly every 6 hours and 1 gm of sulfadiazine administered orally every 4 hours. Treatment with both drugs should be continued for at least 2 weeks. Subsequently, Eisele and McCullough (14) recorded the successful therapy of a seriously ill patient with the combination when failure followed previous treatment with either drug alone. Hall and Spink (4) had treated with streptomycin a patient having subacute bacterial endocarditis due to *Br. abortus*. This therapy was accompanied by definite clinical improvement in the patient's condition, but after he had received 118 gm of streptomycin over a period of 31 days, colonies of *Br. abortus* highly resistant *in vitro* to streptomycin were isolated from blood cultures. One type of colony ("resistant") grew in concentrations of 50,000 units/ml of streptomycin, whereas another type of colony ("dependent") required the presence of streptomycin for growth on agar plates. The patient was then given large doses of sulfadiazine, which eliminated the bacteremia. He expired suddenly because of acute cardiac failure. Postmortem studies showed the presence of a severe myocarditis and bacteria-free vegetations on the aortic valve. Extensive bacteriological studies failed to reveal the presence of Brucella in the tissues or body fluids. This case is presented in some detail because it is the only reported case from which streptomycin-resistant Brucella have been isolated as a result of treatment, and the sequence of events suggested further experimental and clinical studies on the antibrucella effect of streptomycin and sulfadiazine in combination.

Preliminary studies carried out at the University of Minnesota with experimentally infected chick embryos showed that either streptomycin or sulfadiazine prolonged the life of the embryo (5, 6). But unless high concentrations of either drug were used, Brucella organisms were isolated from the viable chicks' tissues. When streptomycin and sulfadiazine were administered simultaneously, prolongation of life not only occurred, but in a high proportion of the embryos, no viable Brucella cells could be isolated from the tissues. In this manner, the combined use of the two in eggs was found to be effective against all three species of Brucella. Subsequently, Shaffer and Spink (15) pointed out that the combined action of these two agents was synergistic, that is, the total effect of both drugs against Brucella was greater than the sum of the two drugs taken independently.

During a period of 2 years almost fifty cases of either acute or chronic brucellosis have been treated with streptomycin and sulfadiazine at the University of Minnesota Hospitals. That there might be no doubt about what clinical condition was being treated, therapeutic evaluation has been based only upon sixteen patients from whom Brucella organisms have been isolated. All cases were due to *Br. abortus*. The patients had been ill for periods ranging from a week to 9 months. When material is selected so

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that only those patients with demonstrable bacteremias are included in the final analysis, it becomes obvious that the more severe cases have been treated, and a therapeutic regime is put to a stringent test. The cases treated included patients with complications such as subacute bacterial endocarditis and involvement of bone (11, 13). Of the eighteen patients, four had a clinical relapse after the completion of one course of therapy. These four patients recovered eventually, either after further specific treatment or spontaneously with the passage of time. As far as infections due to *Br. abortus* are concerned, it would appear that approximately 75 per cent of the patients have had no further symptoms after one course of treatment. Careful bacteriological studies were carried out after the completion of treatment, and there were two additional patients who were asymptomatic but whose blood cultures yielded *Br. abortus*. For purposes of strict clinical investigation these patients have been considered as therapeutic failures. This means that about one-third of the patients treated had either a bacteriological or clinical relapse, or both, following the completion of the first course of therapy. Though this leaves much to be desired, it represents a definite advancement in the specific therapy of human brucellosis.

It is generally agreed that *Br. melitensis* causes a more severe type of infection than does *Br. abortus*. A cooperative study of melitensis infections was made possible between the University of Minnesota group and Dr. M. Ruiz Castaneda, Director of Brucellosis Research in Mexico City. Patients having a demonstrable bacteremia were treated at the Mexico General Hospital. Many of these patients had been ill for several months, suffering from severe neurological complications, and an occasional male had orchitis. Again, since only bacteremic cases were selected for study, the more severe patients were treated. A total of forty-eight patients completed one course of treatment. Of these, 24 recovered completely, that is, clinically and bacteriologically. The remaining patients have had either a clinical or a bacteriological relapse, or both. It is of interest, though, that some clinical improvement was observed in many of the patients classified as therapeutic failures. It may be stated, then, that about 50 per cent of patients having infections due to *Br. melitensis* recover completely following one course of therapy.

Available data in this country would indicate that infections due to *Br. suis* do not respond quite so well to streptomycin and sulfadiazine as to *Br abortus* infections, but they respond better than do infections caused by *Br. melitensis*.

Although there has been some variation in the schedules of doses used, the following schedule was finally selected: 0.5 gm of streptomycin is given

intramuscularly every 8 hours for 14 days. When treatment with streptomycin is started, 3 to 4 gm of sulfadiazine is administered orally and then 1 gm every 4 hours for 14 days. One of the doses of sulfadiazine may be omitted at night. Rather than give larger doses or continue therapy for a longer period, it is recommended that treatment should be discontinued at the end of 14 days. Then, if a relapse should occur, treatment may be given for another 14 days.

Undesirable side effects have been associated with the administration of streptomycin. Experience at the University of Minnesota has revealed that about 5 per cent of patients receiving 0.5 gm of streptomycin every 6 to 8 hours for 2 weeks will develop a marked vestibular dysfunction due to damage to the eighth nerve. For reasons that are not too clear, the incidence of eighth-nerve dysfunction in the patients treated in the Mexico General Hospital was around 15 per cent. No serious untoward reactions were associated with sulfadiazine. Because vestibular dysfunction may persist, considerable caution must be exercised by the physician before treating a patient for brucellosis. Furthermore, such treatment is not inexpensive, since the patients should be hospitalized.

It is not clear why bacteriologic failures occur in patients who have received the combination of streptomycin and sulfadiazine. At the University of Minnesota Hospitals this failure has not been associated with the appearance of streptomycin-resistant strains of Brucella. Comparative studies carried out by Dr. Ellard Yow at the University of Minnesota with five strains of *Br. abortus* isolated from the blood of patients after the cessation of streptomycin-sulfadiazine therapy have shown that these strains are just as sensitive *in vitro* to streptomycin as were the cultures obtained from the patients before treatment was instituted. Through the courtesy of Dr. Sylva of the Mexico General Hospital, Dr. Yow has also studied seven strains of *Br. melitensis* obtained from patients after the discontinuation of therapy, and in only one instance was the post-therapy strain more resistant than that recovered before treatment. The most likely explanation for the failure of streptomycin and sulfadiazine to eradicate Brucella from the body may be correlated with the intracellular parasitism displayed by Brucella organisms. It is not unlikely that a few of the bacteria remain protected against the drugs within the confines of body cells, where they may propagate and eventually find their way into the blood stream.

Dihydrostreptomycin apparently causes less neurotoxic reactions than does streptomycin. Dr. Ellard Yow and Dr. Robert Magoffin at the University of Minnesota have shown *in vitro* and in the experimentally infected chick embryo that, weight for weight, dihydrostreptomycin possesses just as much antibrucella activity as streptomycin. It is now recom-

mended that in the treatment of human brucellosis, dihydrostreptomycin be administered instead of streptomycin.

Conclusions

Human brucellosis may be due to one of three species of Brucella, namely, *Br. abortus*, *Br. suis*, or *Br. melitensis*. In general, milder forms of the disease are caused by *Br. abortus*, and a more severe type of illness results from *Br. melitensis*. Infections due to *Br. suis* are more serious than those caused by *Br. abortus*, but less so than melitensis disease. Clinical observations at the University of Minnesota Hospitals over a period of 2 years have revealed that a combination of streptomycin and sulfadiazine will result in a clinical and bacteriologic recovery in about two-thirds of the cases. The remaining one-third may have a clinical relapse, or blood cultures may show a persistent bacteremia in the absence of symptoms Included in a majority of these "therapeutic failures" are patients who are definitely improved by the treatment. Approximately 50 per cent of the patients with infections due to *Br. melitensis* recover clinically and bacteriologically following treatment. Again, most of the patients considered as failures in treatment are definitely improved as a result of therapy. The combination of streptomycin and sulfadiazine has been helpful in controlling severe complications such as bacterial endocarditis due to Brucella, osseous lesions, and neurological lesions. Because of the neurotoxic reactions induced by streptomycin, especially vestibular dysfunction, it is recommended that instead of streptomycin, dihydrostreptomycin be administered along with sulfadiazine.

A though streptomycin and sulfadiazine have not completely solved the problem of specific therapy, the introduction and subsequent use of streptomycin along with sulfadiazine has provided considerable encouragement in the efforts to treat human brucellosis

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CHAPTER 25

PLAGUE

Until recently, curative medicine found itself powerless against the rapidly disintegrating forces at work in the system of a human being infected with a virulent plague bacillus. The hopelessness of the situation was reflected in Heiser's gloomy statement that there was no specific for plague and in Wu Lien-Teh's remark in reference to pneumonic plague that "no form of treatment was found of any avail" (1). Early in 1940 this dark picture was brightened by the remarkable successes achieved by the therapeutic application of new compounds in the sulfonamide series. In an epidemic in India it was found that the case mortality percentage for septicemic plague was reduced from 92.3 per cent to between 20 and 30 per cent when sulfadiazine was administered early in the course of the disease (2, 3). With the discovery of penicillin, anticipation was high, but it was found that this antibiotic had no therapeutic value under the circumstances of controlled experimental infection (4, 5). Consequently, it was indeed gratifying to record, in July 1944, that streptomycin[1] exerted, both *in vitro* and *in vivo*, a surprisingly powerful antibacterial action on *P. pestis*. Subsequent detailed laboratory studies by Quan, Foster, Larson, and Meyer (6), Hornibrook (7), and others (8, 9) amply confirmed the early observations which led to the conclusion that streptomycin is the most effective therapeutic agent thus far discovered for treatment of bubonic, septicemic, and pneumonic plague infections in mice and guinea pigs. Clinical observations by Videla (10) in Argentina, Karamchandani and Sundar Rao (11) and others (13, 14) in India, and Estrade (15) in Madagascar have since substantiated this conclusion. Their use of this antibiotic cut the well-known high case mortality rate of 75 to 90 per cent to 4 per cent in bubonic and to 10 per cent in septicemic plague.

ANTIBACTERIAL ACTIVITY OF STREPTOMYCIN ON P. PESTIS IN VITRO

Hornibrook reported that growth of *P. pestis* in broth is inhibited by streptomycin in a dilution of 1:160,000 (1.25 μg/ml). The antibiotic

[1] Kindly furnished by Dr. Selman A. Waksman

appeared to have a bactericidal, rather than a bacteriostatic, action according to Herbert. With a concentration of 10 μg/ml, an inoculum of 1,900,000/ml of *P. pestis* (North African human strain 337) was reduced to 1,520/ml in 2 hours and was completely sterilized in 5.5 hours. Quan *et al.*, after more extensive studies on the dynamic action of streptomycin on the plague bacillus, made the following observations: Although the chemical composition and the pH of the suspending medium and the age and density of the culture exert some influence, it is the active concentration of the antibiotic which determines the time necessary for bactericidal action, when the aforementioned factors are kept constant. Under suitable conditions, 1,250 μg/ml of streptomycin in hormone broth kills 100,000,000 virulent *P. pestis*/ml (human strain "Shasta") in 15 minutes. The same number of organisms are destroyed in 4, 12, 24, 48 and 120 hours, respectively, by 313 μg/ml, 78 μg/ml, 39 μg/ml, 20 μg/ml, and 5 μg/ml. With an inoculum of 100,000 organisms of a recently isolated human strain (Modoc), the bacilli were killed by 0.2 μg/ml in 72 hours, whereas to kill an equal number of the Shasta strain required 1.9 μg/ml. Amounts varying from 0.4 to 4.0 μg/ml proved bactericidal to six plague strains of Hawaiian, Egyptian, Indian, and Californian (U.S.A.) origin in 5 days. Avirulent strains are usually more resistant; for example, in 5 days, 100,000 of the E. V. 76 plague bacillus (Girard)/ml are killed only in the presence of 16 μg/ml, strains 14 and 1122 (Jawetz and Meyer) by 8 μg/ml, and the Tjiwidej strain (Otten) by 4 μg/ml. Furthermore, in the presence of 10 per cent blood serum, 40 μg/ml was required to kill 10,000 virulent *P. pestis* (human strain "Yreka") in 48 hours.

In vitro studies in the laboratories of the Hooper Foundation showed that some plague bacilli have the same ability to resist streptomycin as have other types of bacteria. It was found that although the ratio of the slightly resistant mutants varied from strain to strain, usually only few highly resistant bacilli were present in a total of several hundred billion organisms. The biologic behavior of these resistant mutants, however, was found to be the same as that of the parent strains.

Generally, dihydrostreptomycin was found to be slightly less active *in vitro*, as well as *in vivo*, than unaltered streptomycin. An exception was found when a few strains which resisted 5,000 μg/ml of streptomycin could survive only 2,500 μg/ml of the hydrogenated derivative.

Curative Action of Streptomycin in Experimental Plague Infections of Mice and Guinea Pigs

Infections brought about by subcutaneous inoculation with *P. pestis* produces in mice a reasonably close replica of natural bubonic plague in man. When 100 to 1,000 multiples of the M.L.D. are used, the infection

in mice usually becomes generalized, unremitting bacteremia is established in 40 to 60 per cent of the animals, and the immunity mechanism becomes partly damaged by toxins in 36 to 48 hours. At this stage of the infection, repeated use of either highly potent antiplague sera or sulfonamides saves an average of only 35 per cent of the mice, whereas streptomycin in the dose of 500 μg every 3 hours for 3 days or a total of 12.0 mg cured 100 per cent. A smaller total dose of 3.2 mg of streptomycin per mouse injected in amounts of 100 μg at 6-hour intervals for 192 hours invariably sterilizes the blood stream, spleen, and liver; but the bacteria persist in the lymph nodes, and ultimately 40 per cent of the treated mice relapse and succumb to the disease. Relapses are seldom encountered when the dose is increased to 200 μg every 6 hours for 5 to 10 days. When only one large dose is injected intraperitoneally on the 48th hour of the experimental bubonic infection, the median curative dose is 1,000 to 1,250 μg for a 20-gm mouse or 50 to 62 μg/gm. It, therefore, appears that the larger the dose of streptomycin administered, the greater the possibility for the antibiotic to diffuse into the necrotic tissues of the regional bubo in a concentration sufficient to inactivate the large number of plague bacilli in these sites which could readily cause fatal septicemia if treatment were discontinued. This idea has found further support in the tests reported by Sokhey and Wagle (12) in which treatment was instituted on the 72nd hour with 0.8 mg. This amount was given four times a day for 2 days thereafter, and then 0.2 mg was injected four times a day for the next 5 days. A total of 10.4 mg of streptomycin/mouse cured nine of ten mice so treated. A few mice infected with a very small dose of *P. pestis* (48 to 144 organisms) were also treated beginning on the 48th hour after inoculation. Streptomycin in a total dose of 46.4 mg was administered subcutaneously following the same schedule; as might be expected a l the mice so treated were cured.

An experimental disease similar in severity to that in mice is present in guinea pigs on the 120th hour after subcutaneous injection of 1,000 multiples of the M L.D. of *P. pestis*. With a dose of 20,000 μg/kg or approximately 10 mg daily per animal, over a period of 10 days, 80 to 100 per cent of the animals in such a mild stage of septicemia are cured. It requires less streptomycin, therefore, to cure guinea pigs with mild septicemia than to cure mice, on the basis of micrograms of antibiotic per gram of body weight. Wayson and McMahon (8) saved the lives of thirty-eight of thirty-nine guinea pigs with bubonic plague by using a total of 39 to 125 mg of the antibiotic. They commented on the persistence of living plague bacilli in the purulent buboes of nine animals sacrificed on the 21st day after inoculation, or 10 days after apparent recovery. Quan *et al.* showed that injection of 12.5 mg of streptomycin in 0.5 cc of physiological saline solution into the tissues surrounding the bubo at 12-hour intervals for 10 days sterilized the

lesions completely. The antibiotic available at that time [1945] proved very irritating, and even though the buboes became sterile, the necrotic areas were much larger than those of the controls or of the animals treated systemically. The experiment was later repeated with pure streptomycin, which gave rise to no irritation and confirmed the *therapeutic efficiency of local application*.

Unpublished observations by Quan and Meyer (5) failed to reveal a true synergistic action of streptomycin with the sulfonamides. Whenever the highly potent antiplague rabbit gamma globulin solution (new antiplague serum) was administered with streptomycin, however, the percentages of cures in mice were slightly higher. In some ways the antiplague serum furnishes part of the immunity mechanism, which, in advanced infections, is usually severely damaged. Other experiments indicated that a partial immunity induced by previous inoculation with plague antigens potentiates the action of streptomycin in both mice and guinea pigs. Thus, prophylactic immunization of human beings could be expected to increase the chances of recovery from a severe plague infection with the aid of streptomycin.

The maximal effectiveness of streptomycin has been studied with the infection in mice as a model, for this resembles clinical septicemic plague induced by direct blood stream infection through multiple fleabites. To produce this model, slightly resistant mice were injected intraperitoneally with 2,000 virulent *P. pestis*; bacteremia and toxemia exist in such mice by the 9th to 12th hour after infection, and death takes place in 34 to 72 hours. If treatment was instituted by the 9th hour after infection, two doses of 800 μg each, or three doses of 400 μg each at 3-hour intervals were required to cure 80 to 90 per cent of nonbubonic septicemic plague infections. Smaller doses merely delayed death. If treatment was postponed until the 18th or 24th hour, 1,600 μg cured only 25 to 45 per cent of the mice. *Only early treatment with large doses of streptomycin cures septicemic plague in mice.* Under identical conditions, even combined therapy with sulfadiazine and antiplague serum proved ineffective.

Lobular focal pneumonic plague induced in mice or guinea pigs by intranasal instillation or by inhalation of an infective cloud, is anatomically recognizable by the 36th hour and has been effectively cured with streptomycin. The therapeutic dose required to cure 90 to 95 per cent of mice infected for 36 hours varied between 200 and 400 μg every 6 hours or between 6 and 16 mg over a period of 6 to 10 days. Smaller doses or further delay in treatment reduced the chance for cure. The bactericidal action of streptomycin on experimental pneumonic plague in mice is fully illustrated by comparative bacteriological counts of the entire lung tissue of treated and untreated mice (fig. 68). Mice are killed at various intervals, and the lobes of the lungs are removed aseptically and emulsified in a War-

ing blender. Suitable dilutions are plated on rabbit blood agar. Only 6 hours after treatment with doses of 200 μg was started, the number of plague bacilli was reduced from 3,000,000 per mouse lung to approximately 60,000, whereas in untreated animals it had advanced from the same point to 10,000,000. By the 12th to 24th hour of treatment, the spleen of the treated mice became sterile, and less than 500 organisms were counted in the lungs. By the 96th hour after infection, when all untreated mice had died, the lungs and bronchial lymph nodes of treated mice either were sterile or contained only a few thousand plague bacilli in the abscess-like patches of pneumonia. It was impossible to isolate plague bacilli from

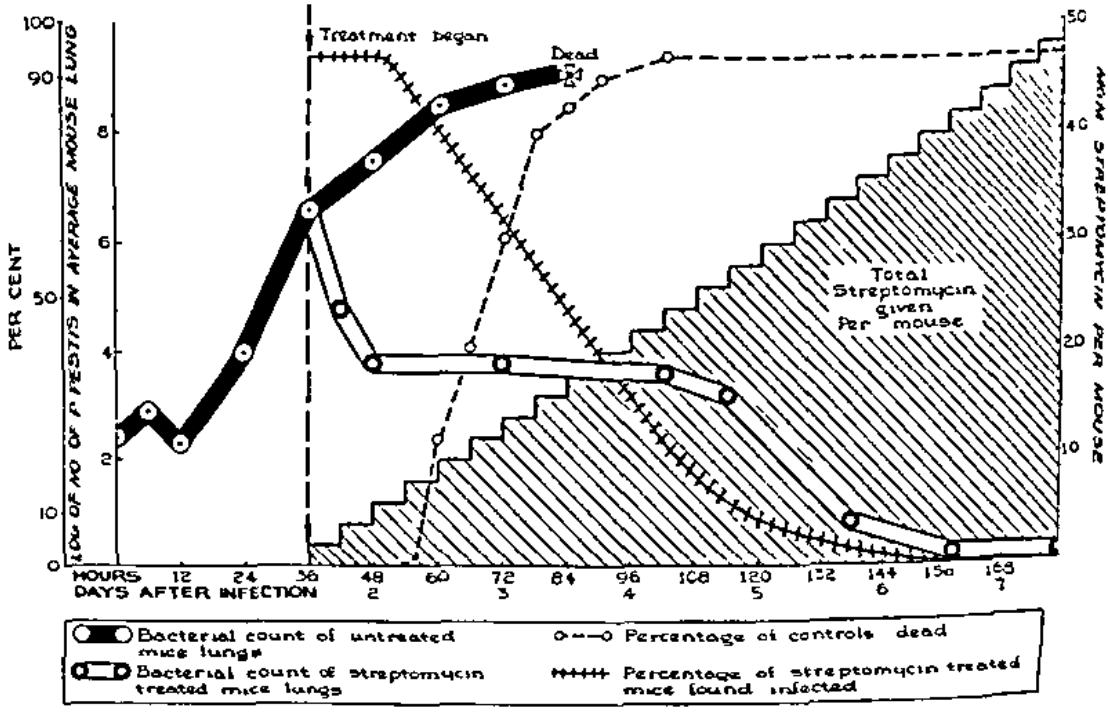

FIG. 68 Bacteriological study of intranasally-infected streptomycin-treated mice

lungs or lymph nodes 100 hours after treatment with streptomycin had been initiated A relatively small amount of streptomycin (5 mg per animal—approximate y 40 μg/gm body weight a day) accomplished these remarkable therapeutic effects In guinea pigs, pneumonic plague lesions produced by intranasal instillation and containing between 1,200 and 6,200 million *P. pestis* on the 36th hour are completely sterilized with a total of 125 mg per animal (weighing about 500 gm) after 6 days of treatment with the antibiotic However, to sterilize lesions produced in guinea pigs which inhaled an aerosol of plague bacilli, required 10 days of treatment with 10 mg/kg every 6 hours, beginning on the 3rd day (fig 69).

The remarkable feature of streptomycin therapy in experimental plague infections of mice and guinea pigs is the success achieved with compara-

tively short periods of treatment, or even with a single dose (16). The therapeutic picture contrasts sharply with that of the sulfonamides, which until recently were the only agents of value in plague therapy. The optimal therapeutic dose of streptomycin in the highly susceptible mouse or guinea pig corresponds to 10 mg/kg of body weight every 6 hours (approximately 3.0 gm a day for an adult weighing 75 kg).

A recent observation, however, has demonstrated that some plague bacilli may resist the action of streptomycin. A guinea pig that had inhaled a

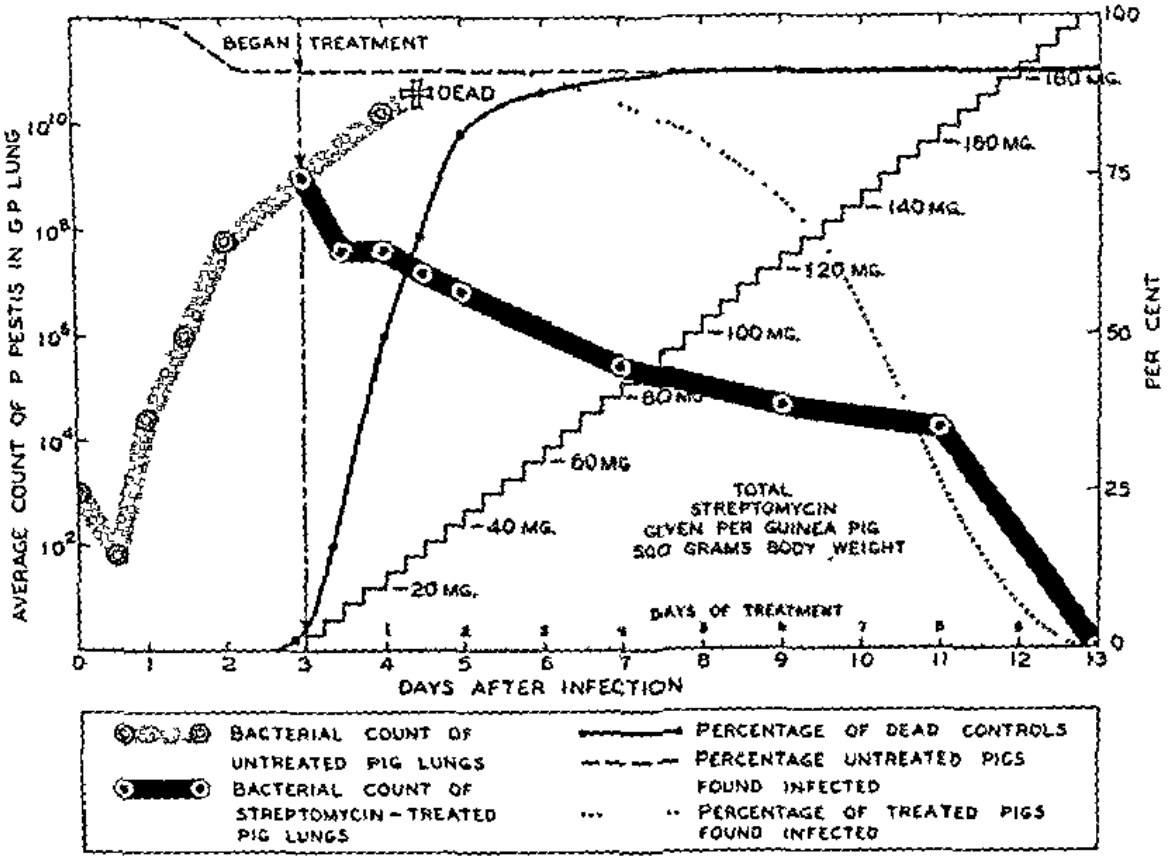

FIG 69 A study of streptomycin treatment of experimental primary pneumonic plague in the guinea pig (infected by inspiration of bacterial cloud).

dense cloud of virulent organisms was treated 72 hours after infection and every 6 hours thereafter with 20,000 μg of streptomycin per kilogram of body weight. On the 6th day of treatment the animal died. *P. pestis* was isolated from the spleen and *grew normally in the presence of 25,000 μg of streptomycin per milliliter of broth.* This strain, still virulent, has biologic characteristics similar in all respects to the parent strain, which grew well only in broth containing less than 4 μg/ml of streptomycin.

The development of streptomycin-resistant plague bacilli must therefore be recognized, particularly when advanced infections are treated with suboptimal doses of the antibiotic. Fortunately, in the treatment of the individual patient this resistance is of minor importance, since it is independent

of resistance to sulfonamides. Collateral therapy with sulfadiazine inhibits the streptomycin-resistant strains until the immunity mechanism of the infected host is fully developed. Equally, the epidemiologic importance is insignificant, since drug-resistant strains are, as a rule, confined to the closed necrotic lesions, and the infection does not become generalized. The bacilli enter the blood stream in numbers insufficient to infect fleas, and thus the transmission chain cannot be established.

Treatment of Human Plague

Bubonic plague

Successful clinical treatment of bubonic plague with streptomycin was first reported in 1947 by Videla in Argentina, who used the drug in six cases of severe bubonic-septicemic plague. One, in addition, was complicated by plague meningitis. Two of the patients were youngsters (14 and 16 years old); the others were adults. Treatment was varied and comprehensive: The patients received sulfamerazine in large doses and antiplague serum (100 ml intramuscularly in adults; 70 ml in children) for 3 days. Penicillin was used whenever pulmonary complication due to secondary infection was feared. The availability of streptomycin afforded an opportunity for its trial when treatment then in use did not appear to be effective. In the complex case of meningeal plague the patient, a 14-year-old girl, received a total of 16 gm of streptomycin intramuscularly and subcutaneously in 5 days. On the 2nd day of treatment 225 mg of the antibiotic was also administered in two doses by the cisternal route and 4 gm by the intramuscular route. Fifteen hours later the purulent rachidian cephalic fluid was proved sterile. The temperature and pulse became normal after 3 days of treatment with streptomycin. Three of the other patients were treated with a total of 9.5 gm in daily doses of 1, 2, 3, 3, and 3, 2.5, 2.5, and 1.5 gm intramuscularly or subcutaneously, beginning on the 3rd to 5th day of sickness In one of the least complicated cases, the patient (29 years old, sterile blood culture on admission) was given 7 0 gm in 4 days from the 3rd to the 6th day of illness. An additional case is found in a report, dated January 1947, submitted by the American Embassy in Buenos Aires to the Secretary of State. In this case of uncomplicated right inguinal bubonic plague, treatment with 17 gm of streptomycin alone was successful. After administration of 12 gm, the temperature fell from 40°C on the 2nd day to 37.5°C on the 5th

obtained by punc

yielded *P. pestis*,

and after the antibiotic treatment. Videla rightly concluded that effective treatment in six rather complicated cases is inadequate to establish the value of any drug, but the results do hold hope for streptomycin.

Another report of the clinical use of streptomycin for plague was made by Karamchandani and Sundar Rao (11), whose case records and temperature charts are of interest for the following reasons: (a) Five patients were all desperately ill, unconscious, and had temperatures of 103° to 106°F. (b) The diagnosis of plague had been confirmed by lymph node puncture in every case. (c) Two of the patients had received sulfathiazole twelve times by mouth and 60 to 100 ml of antiplague serum before streptomycin therapy was instituted. (d) Streptomycin in doses of 0.125 gm every 3 hours was administered for 72 to 96 hours. (e) The total dose used for four patients was 4 gm, and for one, 2 gm. (f) Improvement was noted within 36 hours after 1.5 gm had been given. (g) All of the patients made a complete recovery.

In a final report Karamchandani and Sundar Rao (17) analyze 206 cases of plague and compare the efficacy of various treatments. They selected 15 moribund patients for therapy with streptomycin and although the mortality rate was 20 per cent, they express the view that "if there is any specific against plague it is streptomycin." According to their studies, a total of 1 to 2 gm of streptomycin is the minimal effective dose; the greatest total dose is 8 gm in individual doses of 0.5 gm every 6 hours over a period of 4 days.

These cases were among 152 in an outbreak of plague in the Anantapur District of Madras Presidency in which there were sixty-six deaths—a case mortality rate of 43.4 per cent. The mortality rate may have been low in part because some prophylactic inoculations against plague had been carried out in the district prior to and during the epidemic. In fact, it is noteworthy that a 13-year-old boy who received only 2 gm of streptomycin had been inoculated against plague 6 days before admission, when he had a temperature of 105°F, a pulse rate of 156, respirations of 46 per minute, and a painful enlarged left femoral lymph node It is naturally a matter of conjecture how greatly the sulfathiazole influenced the course of the plague infection in these cases. It was demonstrated at the Hooper Foundation by means of experimental studies on mice and guinea pigs that active immunization of the administration of antiplague serum definitely improves the therapeutic value of streptomycin, as well as that of sulfonamides. These laboratory observations should be considered in an interpretation of the astonishing effectiveness of very small doses of the antibiotic.

Streptomycin treatment in 3 cases of very severe bubonic plague during the outbreak in Haifa were reported by Haddad and Valero (12). Since the patients failed to respond to penicillin, sulfathiazole or sulfadiazine, they were given streptomycin in the doses of 200 to 300 mg every 3 hours for from 5 to 12 days, or a total dose of from 16 to 17 gm of streptomycin. A fourth patient not treated with streptomycin died on the 8th day of ill-

ness. Apparently the buboes were not influenced by the late treatment and required surgical drainage to bring about resolution.

Extensive clinical field trials were reported recently by Sokhey and Wagle (13), who have had a great deal of experience of this sort. Since they had previously demonstrated that the most decisive factor in bubonic plague is development of septicemia, they made not only bubo puncture but blood cultures before treatment. They had observed that as long as the lymph nodes prevent the spread of the bacteria into the blood stream and the infection remains localized, spontaneous recovery is apt to take place in a large percentage of cases. But if septicemia develops, the infection proves fatal in more than 90 per cent of the cases.

During the course of an epidemic in a group of villages, some 40 miles away from Poona (Central Division of Bombay, India), bubonic plague was treated with streptomycin in 124 cases. These cases constituted half of a series of 243 unselected cases in which alternating patients were treated with either a sulfonamide (sulfadiazine or sulfamerazine) or streptomycin.

An initial dose of 0.66 gm was given and then 0.33 gm at 4-hour intervals until the temperature remained normal for 2 or 3 days. Thus, in mild bubonic plague without septicemia the total quantity of drug used varied from *4 to 6 gm.* In septicemic infections the total quantity of the antibiotic used varied between *6 and 12 gm,* but in five cases of exceptional severity it was necessary to use as much as *25 gm.* With this dose schedule, the concentration of streptomycin in the peripheral blood, as estimated in a few cases, reached 5 μg in 4 hours, 10 μg in 8 hours, and about 20 or more μg/ml of serum 24 hours later. The over-all striking therapeutic effects are summarized in table 53.

When a mild infection remained confined to the lymph nodes, the temperature became normal within an average of 29 hours. If severe septicemia existed when treatment was begun, streptomycin brought the temperature down to normal in an average of 55 hours. In a case of primary plague pneumonia with signs of consolidation in the lungs and severe leukopenia, sulfamerazine was used at first, but when the nature of the infective process was recognized streptomycin was given The antibiotic was administered at the rate of 0 66 gm every 4 hours for 10 days. Recovery was uneventful.

No serious toxic symptoms were noticed, in two cases a mild temporary psychosis developed, and in one case, dermatitis, which disappeared when use of the drug was stopped.

During the epidemic near Poona ample opportunity was also afforded to compare the therapeutic effectiveness of streptomycin with that of sulfadiazine and sulfamerazine The definitely superior action of the antibiotic is attested by the comparative case mortality figures also presented in the report of Sokhey and Wagle (table 53).

In a brief report, Gupta referred to the use of streptomycin in the Campbell Hospital, Calcutta, in twenty-four cases of bubonic plague with seven deaths. Only in the severe infections were streptomycin injections given in doses of 1 to 3 gm daily for 4 to 9 days. The severity of the infection could be judged by the fact that two patients died within 12 hours after admission. In another paper about plague in Calcutta, Panja, and Gupta (18) also recorded the interesting observation that *P. pestis* was isolated from a bubo of a patient who had been treated with streptomycin for 2 days.

Pneumonic plague

Reports on the treatment of human primary pneumonic plague with streptomycin are limited to the one by Sokhey and Wagle, two in South

TABLE 53

Effect of streptomycin or sulfonamide treatment on clinical bubonic plague (3)

TREATMENT	CASES	DEATHS	MORTALITY
With or without septicemia when treatment was begun			
			per cent
Streptomycin	124	5	4.0
Sulfadiazine	168	16	9.5
Sulfamerazine	149	9	7.9
Controls	165	96	58.1
With septicemia when treatment was begun			
Streptomycin	30	3	10.0
Sulfadiazine	61	3	21.3
Sulfamerazine	22	7	31.8
Controls	91	84	92.3

Africa (19), two in China, and one in Madagascar. The case of proved primary pneumonic plague resulting from laboratory infection described by Huang, Huang, Chu, and Huang (20) was first treated with sulfadiazine and then received over a period of 18 days, a total of 21.4 gm of streptomycin. Recovery occurred after a stormy protracted course, and it is believed that combined therapy with sulfonamides and streptomycin was responsible for this favorable result.

Another confirmed primary pneumonic plague infection of a laboratory technician in China was brought to our attention by Dr. R. Pollitzer (21). The patient recovered uneventfully after bombardment with 84 gm of sulfadiazine, 80 gm of sodium sulfadiazine, 27 gm of streptomycin, 1,100,000 units of penicillin, 1 dose of rabbit immune serum, and 100 cc of Yersin-type serum.

The following observations made by Dr. F. Estrade at Tananariva, Madagascar, are available (*fig. 70*).[2] A 19-year-old Malagasy native was hospitalized for observation on March 27 at 12:30 p.m., since his father and two brothers had died from pneumonic plague shortly before. On admission his temperature was 37.8°C, but by 3 p m. it had risen to 40°C. Streptomycin treatment was instituted. The patient, nevertheless, became restless and complained of thoracic pain on the right side. The conjunctivas were injected, and the pulse was accelerated. The sputum was then slightly rust colored, and by 7 p m. it had become definitely tinged with blood. By 9 p.m. the patient was delirious and depressed, suffered from

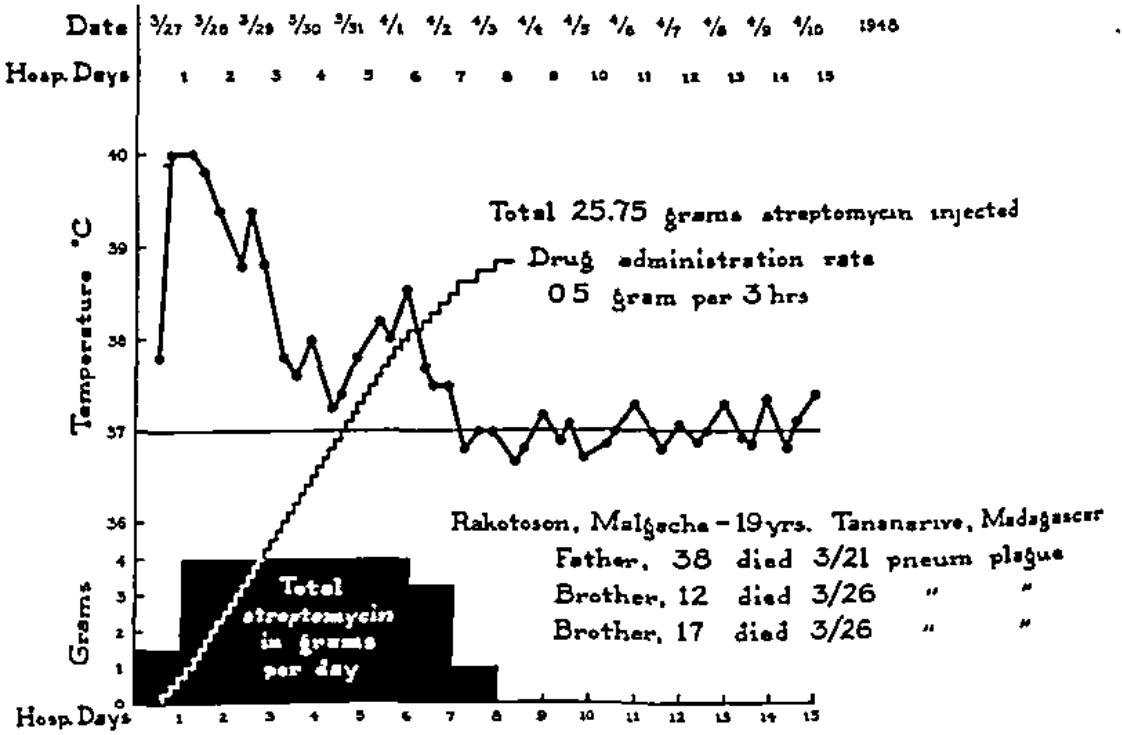

FIG. 70 Primary pneumonic plague streptomycin treated (Observation by Dr F. Estrade, Madagascar).

insomnia, and had a temperature of 40.8°C. The next morning the chest pain extended over a third of the lung; slight consolidation and signs of bronchopneumonia persisted. The sputum became less blood-tinged (rust colored) at 10 a m , and by 4 p.m. was whitish. Although a slight cough still persisted, the patient rested comfortably and his general condition remained good throughout the following day. During a coughing spell at 4:40 p m., however, he raised some whitish sputum containing a small blood clot. Again at 10 p.m. the expectorations were blood-tinged, but the tem-

[2] Through the courtesy of Dr. G. Girard of the Plague Service of the Institut Pasteur.

perature, which had dropped steadily, reached 38.2°C. Progressive improvement was evident on March 30 and 31 with slight temperature fluctuations between 37° and 38°C. A local reaction where the antibiotic was injected in the buttock, which was responsible for the slight rise in temperature, was arrested by local applications of hot compresses. On April 2 and 3 the patient was in excellent condition and had a good appetite Convalescence was uneventful (fig. 70).

When streptomycin treatment was instituted on March 27, the temperature was 40°C; the sputum not only was blood-tinged, but also contained plague bacilli, which was proved by guinea pig inoculation. Every 3 hours the patient was given 0.5 gm of streptomycin; a total of 25.75 gm was administered. In addition, he was given camphostyl, camphor in oil, and drinks containing alcohol. The sputum specimens taken after administration of the antibiotic were proved free from *P. pestis* when inoculated into guinea pigs.

Ordinarily, a person with the symptoms described in primary pneumonic plague would have succumbed within 24 to 36 hours if he had not been given any specific treatment. In reality, the *P. pestis* responsible for the infection was in at least its second human passage, since three other members of the household in which the outbreak occurred had died. In view of these factors, the remarkable effectiveness of streptomycin is uncontestable and deserves the fullest recognition *For the first time in the nefarious history of plague a drug which will cure the pneumonic form has been found.* As soon as this fact becomes generally known, the natives in regions where pneumonic plague occurs will bring the incipient cases to the attention of physicians instead of keeping them hidden in crowded quarters, thus serving as further sources of infection. Then, if streptomycin together with sulfadiazine is used conscientiously and vigorously in the early stages of any outbreak of pneumonic plague, there is every reason to anticipate that it will aid in a rapid termination of the threat of disaster.

Clinical Use of Streptomycin in Human Plague

In regions where plague in wild rodents or in rats is endemic, it is imperative that the possibility of an infection with *P. pestis* be considered constantly by physicians Early diagnosis is of the greatest importance not only to the patient, whose recovery may depend on early administration of streptomycin, but also to his family, his physician, and the community, whose lives may depend on adequate prophylactic precautions. It is recommended that the following procedures be carried out in the order mentioned when the presence of plague is suspected:

1. Administer sulfadiazine or any other effective sulfonamide.
2. Puncture the bubo in its earliest stages with an 18-gauge needle

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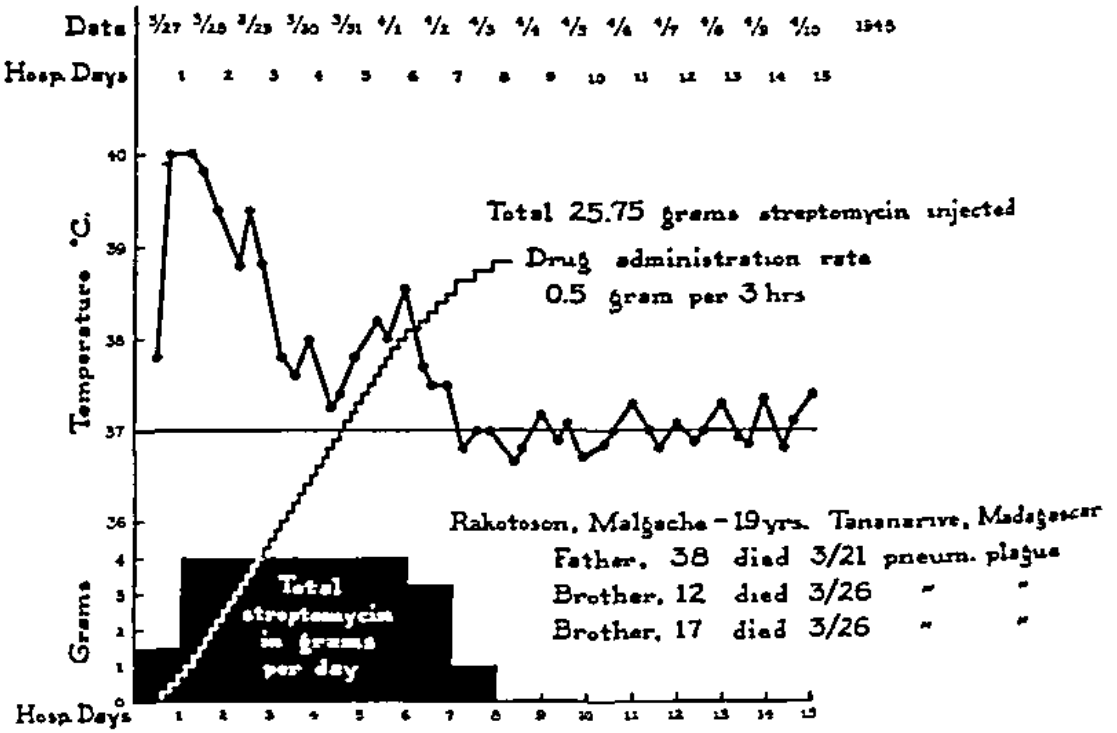

FIG 70. Primary pneumonic plague streptomycin treated (Observation by Dr F. Estrade, Madagascar)

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perature, which had dropped steadily, reached 38.2°C. Progressive improvement was evident on March 30 and 31 with slight temperature fluctuations between 37° and 38°C. A local reaction where the antibiotic was injected in the buttock, which was responsible for the slight rise in temperature, was arrested by local applications of hot compresses. On April 2 and 3 the patient was in excellent condition and had a good appetite. Convalescence was uneventful (fig. 70).

When streptomycin treatment was instituted on March 27, the temperature was 40°C; the sputum not only was blood-tinged, but also contained plague bacilli, which was proved by guinea pig inoculation. Every 3 hours the patient was given 0.5 gm of streptomycin; a total of 25.75 gm was administered. In addition, he was given camphostyl, camphor in oil, and drinks containing alcohol. The sputum specimens taken after administration of the antibiotic were proved free from *P. pestis* when inoculated into guinea pigs.

Ordinarily, a person with the symptoms described in primary pneumonic plague would have succumbed within 24 to 36 hours if he had not been given any specific treatment In reality, the *P. pestis* responsible for the infection was in at least its second human passage, since three other members of the household in which the outbreak occurred had died. In view of these factors, the remarkable effectiveness of streptomycin is uncontestable and deserves the fullest recognition. *For the first time in the nefarious history of plague a drug which will cure the pneumonic form has been found.* As soon as this fact becomes generally known, the natives in regions where pneumonic plague occurs will bring the incipient cases to the attention of physicians instead of keeping them hidden in crowded quarters, thus serving as further sources of infection. Then, if streptomycin together with sulfadiazine is used conscientiously and vigorously in the early stages of any outbreak of pneumonic plague, there is every reason to anticipate that it will aid in a rapid termination of the threat of disaster.

Clinical Use of Streptomycin in Human Plague

In regions where plague in wild rodents or in rats is endemic, it is imperative that the possibility of an infection with *P. pestis* be considered constantly by physicians. Early diagnosis is of the greatest importance not only to the patient, whose recovery may depend on early administration of streptomycin, but also to his family, his physician, and the community, whose lives may depend on adequate prophylactic precautions It is recommended that the following procedures be carried out in the order mentioned when the presence of plague is suspected:

1 Administer sulfadiazine or any other effective sulfonamide.

2. Puncture the bubo in its earliest stages with an 18-gauge needle

mounted on a well-fitted 5- or 10-cc syringe, and aspirate a small smount of gelatinous edema fluid. Spread drops of the exudate on two blood plates and in thin films on slides. Polychromatic stains reveal the morphologic characteristics of *P. pestis*.

3. Collect 0.5 ml of blood from the cubital vein and allow 0 25 ml to run over each of two blood-agar plates or agar slants. These blood cultures are used to detect plague septicemia. The presence of more than ten colonies indicates established severe septicemia, whereas fewer may be interpreted to be temporary showers indicative of mild septicemia.

4. Determine the appropriate dose of streptomycin and begin treatment as soon as possible. The formation of an optimal dose of streptomycin required to cure plague in its different stages and forms depends on further clinical experience. In relatively mild bubonic infections, on the first day 0.25 gm should be given intramuscularly every 4 hours (1.5 gm during the first day) and thereafter every 6 hours until the temperature remains normal for 3 days. In severe septicemia, the initial dose should be high—between 0.50 and 0.75 gm every 4 hours during the 1st and 2nd days; smaller doses then may be administered. Continue treatment for at least 4 or 5 days, by which time the temperature usually has reached normal levels In cases of exceptional severity, particularly in pneumonic plague, an initial dose of 4 to 6 gm daily for 2 days is recommended; for the following 6 days daily doses of 3 or 4 gm should be used. The doses of the antibiotic should be large at first, but for economy they may safely be reduced on the 3rd or 4th day of therapy. After the 5th day streptomycin may be dropped, but sulfadiazine should be continued. In the treatment of plague, the following tentative total maximum quantities of streptomycin are recommended: bubonic plague without septicemia, 4 to 6 gm; septicemic and pneumonic plague, 12 to 25 gm.

When the patient does not respond to the streptomycin-sulfonamide treatment in 2 or 3 days, even when optimal or larger initial doses have been given, the possibility that the infecting strain is resistant to this antibiotic should be considered. In such cases aerosporin, chloromycetin, aureomycin, and potent antiplague horse serum or rabbit antiplague gamma globulin solution may prove beneficial.

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CHAPTER 26

FRIEDLÄNDER'S BACILLUS (KLEBSIELLA PNEUMONIAE) INFECTIONS: THEIR TREATMENT WITH STREPTOMYCIN

The isolation and perfection, during the last decade especially, of new chemotherapeutic and antibiotic agents, which are specific for different infections, have resulted in remarkable improvement in the therapy of many of the infectious diseases. The availability of a number of new compounds, many of which may be therapeutically effective in different degrees, depending on the causative microorganisms, has increased the necessity for accurate bacteriologic diagnosis. Also, the selection of the most effective chemotherapeutic or antibiotic agent complicates rather than simplifies treatment of the infectious diseases. Adequate therapy requires special knowledge and information concerning a number of factors which materially influence its application and the ultimate prognosis, namely:

1. Complete diagnosis, natural course, and immediate and remote potentialities of the disease.
2. Isolation and identification of the responsible organism or organisms.
3. Selection of the most effective, yet least toxic, chemotherapeutic or antibiotic agent available.
4. Use of the optimum daily dosage feasible with avoidance of irreversible or serious toxic effects.
5. Continuation of treatment sufficiently long to achieve arrest or cure of the disease, but withdrawal of therapy with sufficient promptness to avoid drug-fast organisms.
6. Possibilities of other therapeutic agents, of combined chemotherapy, and use of surgery in combination with chemotherapeutic agents or antibiotics (Surgery is usually necessary if localized collections of pus or abscesses are present, even though the organism is sensitive to the chemotherapeutic agent being used.)

The above problems related to the best choice and use of chemotherapeutic or antibiotic agents and the timely integration of surgery are often difficult but usually not unsurmountable. The availability of several po-

tentially effective agents affords a novel opportunity of designing the specific therapy to the individual needs of the patient.

The isolation of streptomycin in 1944 by Schatz, Bugie, and Waksman (1) and the demonstration by them, later confirmed by numerous others, that this drug inhibits the *in vitro* growth of a wide variety of gram-negative organisms, including Friedländer's bacillus (*Kl. pneumoniae*) and other microorganisms such as *M. tuberculosis*, added still another promising therapeutic agent to the rapidly increasing number of compounds available for the treatment of infectious diseases. *In vitro* tests of streptomycin were soon followed by *in vivo* studies and later by clinical trials, at first on a limited but more recently on a vast scale.

Heilman (2) was one of the first to test, in 1945, the effect of streptomycin on the growth of *Kl. pneumoniae in vitro*. He tested nine different strains. Five of the nine strains had been isolated only a short time before his studies. Four other strains, which had been subcultured repeatedly, were also tested by Heilman for streptomycin sensitivity. All four strains belonged to type A. Although there was some difference in the sensitivity of the nine strains, all proved sensitive *in vitro* to 1.5 to 2.5 units of streptomycin per milliliter of culture medium. All growth was completely inhibited by these concentrations of the antibiotic.

In vivo tests, also by Heilman, indicated a marked protective effect of streptomycin for mice infected intraabdominally with three different strains of organisms. Sixty-nine mice, inoculated with 1,000 to 10,000 times the lethal dose of *Klebsiella* organisms, were treated with streptomycin for 2 to 3 days. Of those treated, 90 per cent survived But of forty-nine mice, similarly infected and used as controls, all died. It was found however that, when mice were infected by the intranasal route, larger daily dosage of streptomycin and more prolonged treatment were necessary to achieve effective protection. The results of these *in vitro* and *in vivo* studies strongly indicated that streptomycin might prove therapeutically effective in the treatment of Friedlander's bacillus infections in man. Before streptomycin therapy of clinical Friedlander infections is considered, however, certain pertinent aspects of the disease itself should be discussed briefly.

Infections Caused by the Bacillus

In order more accurately to evaluate the therapeutic efficacy of a new chemotherapeutic agent or antibiotic, such as streptomycin, particularly in the treatment of such infections as those caused by *Kl pneumoniae*, it is essential to bear in mind certain facts concerning the organism and certain characteristics of the wide variety of pulmonary and extrapulmonary diseases caused by the organism.

Incidence and distribution of the organism

Ford (3) has reported that Friedlander's bacillus is widely distributed in nature. It has been shown to exist in the air, in soil, and in large bodies of water. Man is a frequent carrier. Dudgeon (4) pointed out that the organism has been demonstrated in the intestinal tract of 5.5 per cent of normal persons, and Bloomfield (5) found the organism in the respiratory tract of approximately the same percentage of normal individuals.

Principle sites of infection

A review of the literature strongly suggests that prior to a decade ago attention had been primarily focused upon pulmonary infections caused by Friedlander's bacillus. In one of the largest series reported, however, Baehr, Shwartzman, and Greenspan (6), in a study of 198 cases, emphasized that, in their experience, extrapulmonary infections were more prevalent than pulmonary infections. They found that the gastro-intestinal tract was the "primary site" in 31 per cent of their cases, the genito-urinary tract in 25 per cent, the biliary passages and liver in 23 per cent, the lungs and respiratory tract in only 12.5 per cent, miscellaneous sites in 5 per cent, and the vagina, uterus, and adnexa in 3.5 per cent. Their findings suggest that more than 50 per cent of Friedlander's bacillus infections are likely to be located in the gastro-intestinal tract and biliary passages and liver, whereas only about one-fourth of this number is likely to be found in the respiratory tract In a series of 4,310 cases of lobar pneumonia studied at Bellevue Hospital between 1920 and 1931 inclusive, only 33 cases of Friedlander's pneumonia were found (7). On the other hand, it has been estimated that "approximately 1 to 3 per cent of all pneumonias are due to *Kl. pneumoniae*" (8)

Predisposing factors

A debilitated condition resulting from any of a wide variety of causes, such as diabetes and other nutritional disturbances, chronic sinusitis, or interference with drainage in the urinary or biliary tracts, may predispose to Friedlander's bacillus infections It has not been the writer's experience, however, that Friedlander's bacillus infections are found any more often in patients with chronic bronchitis, bronchiectasis, pulmonary tuberculosis, or bronchiogenic carcinoma than in patients without these conditions. It has been noted that poor oral hygiene and carious teeth are frequently present in patients developing Friedlander's pneumonia. Chronic alcoholism also may be a predisposing factor, as it frequently is in pneumococcus pneumonias The disease seems to occur more frequently in infants and elderly persons than in other age groups.

Morbid anatomy and clinical features

It has been demonstrated experimentally (9) that, when mice are inoculated subcutaneously with Friedländer organisms, a rapidly spreading acute suppurative process, containing viscid exudate and consisting largely of leucocytes, fibrin, and bacilli, develops. Likewise, intraperitoneal infection in mice leads to an acute suppurative peritonitis with viscid exudate and rapid appearance of septicemia and death of the animals within a short time.

Reports in most texts concerning the morbid anatomy and clinical features of the disease are sketchy. Boyd (10) stated that Friedländer's "pneumonia runs an acute course"; the lesions are usually lobar, occasionally lobular, and have a characteristic mucinous appearance. Cecil (11) described the disease as a classic type, usually lobar in extent, but multiple lobes may be involved. The lesions are characterized by a sticky viscid exudate and massive consolidation.

In a fatal case of Friedländer's pneumonia (12), inadequately treated with streptomycin (2 days), the autopsy revealed extensive consolidation due to acute inflammatory changes and multiple abscesses or cavities. Microscopic examination showed areas of bronchopneumonia surrounding the abscess cavities. Pure cultures of Friedländer's bacillus grew from pus removed from one of the cavities.

In a streptomycin-treated but fatal case of Friedlander's bacillus meningitis (13), autopsy revealed that death resulted from pulmonary embolism. It is significant however that, although necropsy showed evidence of "nearly healed meningitis," several small abscess cavities, filled with purulent material, were found in the lungs. This finding again emphasizes a fundamental fact often observed in different infectious diseases treated with chemotherapeutic or antibiotic agents; namely, that, although the drug may often cure a usually fatal acute meningitis, it may nevertheless fail to sterilize large collections of pus, such as those found in the pleural space, pulmonary cavities, the gall bladder, and elsewhere.

In two cases of Friedlander's pneumonia, recently treated with streptomycin by the writer, lobectomy was eventually necessary because of persisting cavities and bronchiectasis. Examination of the removed lobes showed a considerable amount of organizing pneumonia and fibrosis adjacent to the small abscess cavities and minimal bronchiectasis. Marked healing had occurred in one of these patients following 5 weeks of streptomycin therapy. Previous extensive consolidation had undergone almost complete healing, largely by resolution, at the time of lobectomy. It is significant, however, that small abscess cavities were still present in the right upper lobe, which was removed several weeks after completion of streptomycin therapy. This case illustrates the remarkable effectiveness

of streptomycin in bringing about rapid resolution of acute pneumonic processes that have not advanced to necrosis and abscess formation. It also emphasizes the *limitations* of streptomycin therapy so far as complete healing and closure of cavities are concerned, no matter whether they are due to Friedländer's bacillus or to the tubercle bacillus.

It is evident that the pathology of Friedlander's bacillus infections varies greatly according to the principal site of the disease, age of the lesions, resistance of the host, virulence of the particular strain of the invading organism, and treatment the patient has received. It is equally clear, from an increasing number of reports, that extrapulmonary Friedländer's bacillus infections are more common than pulmonary infections. Suppuration, abscess formation, and chronicity following an acute phase are also prominent characteristics of the disease regardless of the organ or system involved. These significant pathological and clinical features so characteristic of the disease greatly influence and often predetermine results of chemotherapy. Some understanding of the natural course and chronic potentialities of the disease emphasize the necessity of early and accurate bacteriological diagnosis and the immediate institution of specific therapy.

Prestreptomycin Treatment and Results

Prior to the advent of modern chemotherapy, treatment of *Kl. pneumoniae* infections was ineffective and the mortality was extremely high. In a review of the literature from 1882 (the year Friedlander announced the discovery of the bacillus) to 1938, Hartman (14) found a mortality rate of 94 per cent in 232 Friedländer's pneumonia patients. In 1939 Baehr *et al.* reported a mortality of 17 per cent in patients having urinary tract infections, 30 per cent in those having biliary tract infections, and 75 per cent in patients with Friedländer's bacillus bacteremia.

A wide variety of drugs and other therapeutic measures have been used, generally without success. The use of type II antipneumococcus and later specific serum, which were tried before the sulfonamides and penicillin became available, influenced clinical results little if at all (15).

Reports (16, 17, 18, 19, 20) in recent years on the use of the sulfonamide compounds and penicillin suggest that there has possibly been some reduction in the mortality rate in patients with
(21), in a review of the literature and report
out that he had found reports of thirty-five
meningitis with recovery in six. It appears, however, that two of the six patients received streptomycin. Nevertheless, a number of authors have reported cure of Friedlander's bacillus infections with use of the sulfonamides and penicillin. Despite occasional successes following the use of such combined chemotherapy, often in conjunction with surgery, the mor-

tality rate for the disease remained high. In 1948 Jacob and Top (20) reported on seven cases of Friedländer's bacillus meningitis, in two of which the only chemotherapy received was penicillin and the sulfonamides. Both patients were reported as completely recovered. The remaining five patients received various types of treatment, including the sulfonamides and penicillin. All five died. In 1947 Solomon reported sulfadiazine-penicillin treatment of three patients with Friedländer's bacillus meningitis, with fatal results in all. Thus it may be concluded that prior to the advent of streptomycin the mortality in patients with Friedlander infections, particularly meningitis and bacteremia, remained discouragingly high.

Streptomycin Treatment

The effectiveness of streptomycin in Friedländer's bacillus infections is due to the initial sensitivity of the organism to the action of the drug. Since many bacteria, including Friedländer organisms, are known to become resistant to streptomycin *in vitro* and *in vivo*, it is essential to determine periodically the sensitivity of the pathogen during treatment. Otherwise, streptomycin therapy might be continued uselessly should the organism become drug-fast.

Absorption and excretion of streptomycin

As a result of many studies, it is known that, following parenteral administration of streptomycin, the drug is readily absorbed, diffused, and excreted by the kidneys. Following the intramuscular injection of 1 gm of streptomycin, comparatively high blood serum levels may be found, and a considerable amount of the drug is present in the urine, some in the bile, but virtually none in the feces. Also, after similar administrations of streptomycin to individuals without meningitis, usually only a small amount of the drug can be demonstrated in the spinal fluid. If the patient has acute meningitis, however, considerably greater amounts of the drug may be present in the spinal fluid. Since streptomycin is excreted in large part by the kidneys, it is important to know the status of renal function during the course of streptomycin therapy. Excessive amounts of the drug retained in patients with impaired renal function may cause irreparable damage to hearing and vestibular function. Serious toxic effects can usually be avoided, however, if there is proper supervision of treatment. This should include careful attention to renal function and periodic caloric stimulation and audiometric tests to check vestibular and otic functions.

Dosage, duration, and methods of treatment

There is still wide variation in the recommended daily dosage and duration of streptomycin therapy, not only for Friedländer but for other infec-

tions. It is generally agreed, however, that the most effective method of administering the drug is by intramuscular injection, usually twice every 24 hours Intrathecal treatment is also necessary in patients with meningitis. In general, the type and severity of the infection and the body weight of the patient are important guides in determining the daily dosage and duration of treatment. Since streptomycin has become highly purified chemically, it is possible to administer 20 to 25 mg/kg of body weight daily—approximately 1 0 to 1.5 gm daily for adults—for prolonged periods without undue or irreversible toxic reactions. Dihydrostreptomycin, which is now available and appears equally effective therapeutically, is considerably less neurotoxic than streptomycin. A fairly large number of patients under the writer's supervision have received 40 mg of dihydrostreptomycin per kilogram of body weight daily (2.0 to 3 0 gm daily in adults) for 2 or 3 months, without serious toxic effects.

In patients with Friedländer's bacillus bacteremia, with or without meningitis, relatively large daily dosages of streptomycin (2.0 to 3.0 gm daily in adults) should be given intramuscularly for 6 to 12 days, and perhaps half this dosage continued for an additional 7 to 14 or more days after the acute phase has subsided. Under similar circumstances, children may be given smaller *total* daily dosage but they should receive 30 to 40 mg/kg of body weight. If meningitis is present, 50 to 150 mg of streptomycin should also be given intrathecally to adults for the first few days, followed by smaller amounts of the drug every other day or every third day for varying periods, until repeated cultures of the spinal fluid are negative for Friedlander's bacillus and collateral evidence shows a subsiding or essentially normal condition. Considerably smaller intrathecal doses, that is, 25 to 50 mg, may be adequate for children with Friedlander's bacillus meningitis Children generally tolerate the drug exceedingly well. Precaution should be taken, however, to prevent severe toxic reactions and chemical irritation of the meninges.

In patients less severely ill with pneumonia or involvement of the abdominal viscera but without bacteremia or meningitis, somewhat smaller daily doses (1 0 to 2 0 gm for adults and 0.5 to 1.0 gm for children), administered intramuscularly twice daily, may prove sufficient. Practical guides in the choice of daily dosage and duration of treatment are: type and severity of the disease; clinical response of the patient; bacteriologic findings during treatment, including sensitivity of the organism to the drug; and possible or probable necessity of surgery In many patients showing satisfactory clinical, bacteriologic, and roentgenologic response (22), streptomycin was not necessary for more than 7 to 14 days. Should the infection persist in a subacute or chronic form and the bacteriologic examination remain positive and the organism sensitive to streptomycin, it may be necessary to

continue treatment for considerably longer periods than those indicated. If surgery proves necessary (lobectomy for residual cavity or chelecystectomy for biliary tract infection), it should be performed, if possible, before the organisms become drug-fast. Since resistant organisms may emerge rapidly (23), frequent sensitivity studies should be carried out as a guide in timing surgical intervention.

Discussion and results

Since the therapeutic effect of streptomycin in Friedländer infections is primarily related to the bacteriostatic action of the drug and not to any *direct* effect upon repair and healing, such as resolution, fibrosis, or cavity closure, it is logical that clinical results following use of the drug vary markedly, depending on a number of fundamental factors pertaining to the age, location, extent, and character of the disease when streptomycin therapy is begun, as well as on the capacity of the individual to complete the eradication of the infection and repair the damaged tissues. Striking examples of these basic principles are afforded by the autopsy findings in a streptomycin-treated case of meningitis and pneumonia reported by Tartakoff, Grynbaum, and LeCompte (13), and in the surgically removed lobes of two patients with Friedländer's pneumonia previously treated with streptomycin by the writer In the former case, although the autopsy revealed "nearly healed meningitis," nevertheless it also showed the presence of pulmonary abscesses and cavities. In the two cases of lobectomy performed because of residual pulmonary cavities, serial roentgenograms revealed marked healing by resolution of the acute pneumonic process but comparatively little effect upon the cavitary lesions. These cases are cited to emphasize that the degree of healing following streptomycin therapy varies remarkably, even in the same individual. This variation is primarily related to the factors just mentioned. This is further borne out by the results reported by Keefer and Hewitt in streptomycin-treated cases of chronic urinary tract infections, in which they had their least satisfactory results: of thirteen such cases treated, nine showed permanent improvement, one temporary improvement, two no effect, and one patient died (22).

BACTEREMIA

Another important factor influencing clinical results is bacteremia Keefer and Hewitt reported that, in twenty-seven cases with bacteremia, seven, or 26 per cent, died, whereas thirty-seven patients without bacteremia, only six, or 16 per cent, died. Davis, Cheek, and Harrell (24) reported a case of acute Friedlander's pneumonia with pleural effusion and bacteremia, treated unsuccessfully with penicillin and sulfadiazine prior to successful treatment with streptomycin.

MENINGITIS

Development of Friedländer's bacillus meningitis always carried a grave prognosis. Before streptomycin became available, meningeal involvement was almost universally fatal. In eight streptomycin-treated cases of Friedländer meningitis collected from the literature (13, 21, 22, 26), four died and four are reported as apparently cured (tables 54 and 55).

PNEUMONIA

In forty streptomycin-treated cases of Friedlander pneumonia recently reported in the literature, fifteen of which had bacteremia; of these, five had frank abscess cavities and two pleural effusions, and only seven, or 18 per cent died. Three of the seven patients who died had bacteremia and one had frank abscess. The remaining 82 per cent are reported as permanently improved or cured. Two of the writer's patients, however, required lobectomy because of residual cavities.

GENITO-URINARY TRACT INFECTIONS

Of 17 streptomycin-treated patients with Friedländer infections of the genito-urinary tract, reported by Keefer and Hewitt, twelve showed permanent improvement, two temporary improvement, two no streptomycin effect, and one died.

MISCELLANEOUS INFECTIONS

Fifteen cases of miscellaneous Friedlander infections, treated with streptomycin, have been recently reported and one case has been treated by the writer. Of these, four are dead, eleven are reported as temporarily or permanently improved, and one showed no improvement. Of the four patients who died, one had brain abscess, one multiple metastatic abscesses, one endocarditis, pneumonia, and empyema, and one had endocarditis

It is evident that, in all types of Friedlander bacillus infections treated with streptomycin, the prognosis is far better than in patients not so treated. In a total of 82 Friedländer infections treated with streptomycin by the author and as reported in the literature (12, 13, 18, 19, 21, 22, 24, 25, 26, 27) there were sixteen deaths. According to present standards of streptomycin treatment, several of the patients who died apparently received inadequate streptomycin therapy, either because of the scarcity of the drug or the difficulty of obtaining it promptly.

This over-all mortality rate of 20 per cent in patients treated with streptomycin, in comparison with a mortality rate of 94 per cent in 232 patients not so treated (reported by Hartman), indicates the remarkable effectiveness of streptomycin in the treatment of *Klebsiella* infections. It is believed than even better results may be anticipated with earlier diagnosis and more

TABLE 54

Results of streptomycin therapy of Kl. pneumoniae infections (22)

CLINICAL DIAGNOSIS	NUMBER OF PATIENTS	PERMANENTLY IMPROVED	TEMPORARILY IMPROVED	NO STREPTOMYCIN EFFECT	DIED
		Number	Number	Number	Number
Pneumonia	27	20	2	—	5
Pneumonia and lung abscess	4	2	1	—	1
Pneumonia, postoperative	1	1	—	—	—
Pneumonia and sterile pleural effusion	1	1	—	—	—
(15 of the above pneumonias had *Friedländer's* bacteremia)					
Total pneumonias	33	24	3	—	6
Genito-urinary tract infections:					
Acute	4	3	1	—	—
Chronic	13	9	1	2	1
Total urinary tract infections	17	12	2	2	1
Miscellaneous infections:					
With bacteremia:					
Brain abscess	1	—	—	—	1
Endocarditis	1	—	—	—	1
Endocarditis with pneumonia and empyema	1	—	—	—	1
Localized infection	1	1	—	—	—
Liver abscess	1	1	—	—	—
Mastoiditis	1	1	—	—	—
Meningitis	1	1	—	—	—
Multiple metastatic abscesses	1	—	—	—	1
Total with bacteremia	8	4	—	—	4
Miscellaneous infections:					
Without bacteremia:					
Cholangitis	1	1	—	—	—
Localized infection	1	1	—	—	—
Meningitis	4	2	—	—	2
Total without bacteremia	6	1	—	—	2
Total miscellaneous infections	14	8	—	—	6
Grand Total	64	44	5	2	13

TABLE 55

Results of streptomycin therapy of Kl. pneumoniae infections

AUTHORS	CLINICAL DIAGNOSIS	NUMBER OF PATIENTS	PERMANENTLY IMPROVED	TEMPORARILY IMPROVED	NO STREPTOMYCIN EFFECT	DIED	REMARKS
			Number	Number	Number	Number	
Fisher (21)	Meningitis	1	1	—	—	—	Patient received sulfadiazine and penicillin prior to streptomycin.
Hough and Adelson (26)	Meningitis	1	—	—	—	1	
Tartakoff, Grynbaum and Le Compte (13)	Meningitis	1	—	—	—	1	Patient received sulfadiazine and penicillin prior to streptomycin Received streptomycin 3 days. Autopsy showed death caused by pulmonary embolism; also a "nearly healed meningitis."
Herrell and Nichols (27)	Ozena	4	—	3	1	—	Patients received streptomycin by I M. injection and nebulization
Riggins	Ozena	1	—	1 marked	—	—	Patient received 1 gm streptomycin daily I M. for 3 weeks. Cultures from nasopharynx became neg for Friedlander's bacillus
Bishop and Rasmussen (18)	Pneumonia	1	1	—	—	—	Excellent result
Davis, Cheek and Harrell (24)	Penumonia with bacteremia and pleural effusion	1	1	—	—	—	Patient unsuccessfully with sulfadiazine and penicillin prior to successful treatment with streptomycin.
Learner and Minnich (19)	Pneumonia	1	1	—	—	—	Excellent result

Miller, Orris and Taus (25)	Pneumonia	1	1	—	—	—	19-day-old infant
Vickery and Gray (12)	Pneumonia	1				1	Streptomycin treatment inadequate (2 days)
Riggins	Pneumonia	1	1	—	—	—	Patient received 3 gm streptomycin daily I.M. for 3 days. Streptomycin discontinued because of severe toxic reactions.
Riggins	Pneumonia	1	1	—	—	—	Patient received 2 gm streptomycin daily I.M for 5 weeks. Lobectomy for residual cavities and bronchiectasis
Riggins	Friedlander pneumonia with tuberculosis	1	1	—	—	—	Patient received 2 gm streptomycin daily I.M for 3 weeks Lobectomy because of persisting cavities and sputum positive for Friedlander's bacillus and tubercle bacilli.
Herrell and Nichols (27)	Suppurative disease of tracheobronchial tree	2	—	2	—	—	Patients received streptomycin by I.M. injection and nebulization Marked symptomatic improvement.
Grand Total		18	8	6	1	3	

intensive streptomycin therapy. It is also evident that the most satisfactory and striking results are obtained in patients with early acute Friedländer infections and the least satisfactory results in patients with chronic disease that has progressed to extensive necrosis and abscess formation or in patients with such severe types of infection as meningitis with or without brain abscess, endocarditis, or bacteremia.

Toxicity

The use of streptomycin is frequently followed by certain toxic reactions These may be local reactions at the site of injection; sensitization reactions, such as drug pyrexia, cutaneous rashes, or eosinophilia; histamine-like reactions, such as anorexia, nausea, sweating, headache, lassitude, and, rarely, tachycardia; renal reactions which are usually manifested by the appearance of casts, albuminuria, and occasionally by impaired function; or neurological reactions, the most important of which are loss of hearing and vestibular dysfunction. Various other types of minor toxic effects are frequently encountered.

The more severe toxic manifestations can usually be avoided if renal, otic, and vestibular functions are carefully followed by methods previously suggested.

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intensive streptomycin therapy. It is also evident that the most satisfactory and striking results are obtained in patients with early acute Friedländer infections and the least satisfactory results in patients with chronic disease that has progressed to extensive necrosis and abscess formation or in patients with such severe types of infection as meningitis with or without brain abscess, endocarditis, or bacteremia.

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response noted, treatment was continued for 5 to 21 days, with an over-all average of 8.5 days. The average total dose was 0.85 gm.

Aerosol route

In an individual ill with whooping cough, *H. pertussis* can be cultured only from the respiratory tract. It is conceivable, therefore, that the penetration of aerosol mist might have some effect on the etiological agent. Abramson (4), Bryson and Grace (5), and others believe that the aerosol mist may penetrate as far as the smaller bronchioles in the alveoli of the lung. The dosage of streptomycin, up to 3 years of age, was 50 mg/ml of solution three times daily, or a total of 0.15 gm a day. The duration of treatment ranged from 3 to 25 days, with an average of 8 days. The total dosage per child averaged 1.2 gm. In children over 3 years of age, the

TABLE 56

Total number of children receiving streptomycin according to the method of administration

ADMINISTRATION	AGE		TOTAL
	Under 1 year	Over 1 year	
Intranasal	34	18	52
Aerosol	32	24	56
Intramuscular	26	—	26
Intramuscular and aerosol	40	15	55
Total	132	57	189

solution contained one-third gm of streptomycin, given three times a day, dissolved in 2.5 ml of saline

During the catarrhal stage of whooping cough the trachea and bronchi often contained a sticky tenaceous secretion; aspiration of the posterior pharynx was performed before treatment was begun.

In children up to 3 years of age, a small or medium sized hood was placed over the head. The hood had an ice compartment in the top, two openings in front, and a plastic tube inlet in the rear which normally carried oxygen. A plastic Vaponephrin nebulizer was plugged into one of the openings in front. The other openings were not plugged.

With the oxygen regulator set at 80 per cent and the oxygen running at approximately 4 liters per minute through the nebulizer, a moderate stream of aerosol mist was produced. The oxygen content of the hood during treatment averaged 50 per cent as measured with an electromagnetic oximeter Complete nebulization of the solution required about 20 minutes. At the end of the treatment 0.5 to 2 ml of water was introduced into the

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CHAPTER 27

WHOOPING COUGH

The etiological agent of whooping cough, *H. pertussis*, is sensitive to streptomycin in concentrations of 1 to 3 μg/ml of media and is approximately as sensitive to streptomycin as is *H. influenzae*. Bradford and Day (1) showed that streptomycin exerted a protective therapeutic effect on the course of experimental murine pertussis, as indicated by a significant reduction in the mortality rate and the disappearance of the organism from the lung of surviving mice. The experience here reported deals with the observation of the effect of streptomycin in children ill with whooping cough as observed at the Willard Parker Hospital (2, 3).

MATERIAL AND PROCEDURES

Because of the difficulty of establishing a diagnosis of whooping cough in young children before the occurrence of a typical paroxysm, only a small number of cases were admitted to the hospital before the paroxysm was first noted. Most of the children were admitted during the first week of paroxysms. The majority of children came from families of low economic status, and very few had received any active immunization.

This report discusses the use of streptomycin in 189 patients, of whom 132 were less than a year old. The patients were divided into four main groups according to the method of administration of the antibiotic (table 56) The calcium complex of streptomycin was administered by intranasal drops, aerosol mist, intramuscular injection, and a combination of aerosol and intramuscular injection The four methods of administration were about equally divided in children less than 1 year old. Severity of illness was often a factor in the choice of method

Intranasal route

The dosage of streptomycin was not scaled to age. A solution of 40 mg/ml of normal saline was drawn from the vial by means of a small sterile

The course of the whooping cough was favorably influenced when the streptomycin was administered by aerosol, intramuscular injection, or by simultaneous administration of aerosol and intramuscular injection.

Results and Evaluation

Since whooping cough may have a very variable course, it is difficult to evaluate the effect of any single therapeutic agent. In severely ill infants, medical acumen and expert nursing care are of utmost importance.

TABLE 57

Course of illness in children moderately ill on admission

Therapy and route	Number of patients	Subsequent condition		
		Good	Unchanged	Poor
Aerosol	41	31	7	3
Intramuscular	16	14	2	—
Nose drops	46	33	13	—
Aerosol and intramuscular	22	15	2	5
Total all patients	125	93	24	8

TABLE 58

Course of illness in children severely ill on admission

Therapy and route	Number of patients	Subsequent condition			
		Good	Unchanged	Poor	Expired
Aerosol	6	5	1	—	—
Intramuscular	9	7	1	—	1*
Nose drops	3	2	1	—	—
Aerosol and intramuscular	31	18	3	6	4*
Total all patients	49	32	6	6	5

* Also received serum.

Lives may be saved by prompt and correct judgment in the choice of procedures These procedures include oxygen therapy, aspiration of the pharynx and larynx to clear the airway of tenaceous secretion, parenteral therapy, and a special feeding technique (6).

On admission about two-thirds of the children treated with streptomycin were moderately ill, about one-fourth severely ill, and the remainder were mildly ill.

Table 57 shows that the treatment of those moderately ill had a favorable influence in 75 per cent of the patients and failed in only 5 per cent.

nebulizer, and another 5 to 10 minutes of operation was necessary for this to be nebulized. This addition of water assured us that the patient had received all the streptomycin solution and also prevented clogging of the nebulizer from drying of the original solution.

Special care was taken to maintain the nebulizer in a horizontal position throughout treatment. To prevent the infants from dislodging the nebulizer, it was often necessary to use a mummy restraint. It was found advisable to administer the treatment soon after feedings or at such a time as infants were usually quiet enough not to object to the temporary restraints.

In older children the aerosol was administered by means of a cone made from a discarded roentgen film. The cone was placed over the child's nose and mouth. A few children had the nebulizer placed directly in the mouth.

Intramuscular injection

It is recommended that children under 1 year receive 50 mg every 3 hours for 5 days; those from 1 year to 2 years, 75 mg every 3 hours; and those from 2 to 3 years, 100 mg every 3 hours. Children over 3 years should be given a total of 1 gm of streptomycin in 24 hours, equally divided into eight doses.

Combined aerosol and intramuscular injection

Streptomycin was given by the combined method to fifty-five children. The total amount recommended for the combined method was the usual aerosol dosage plus the usual intramuscular dosage.

CONDITION ON ADMISSION AND SUBSEQUENT COURSE

Patients on admission were graded according to the severity of the disease: (a) A child was considered to be mildly ill when the paroxysms were not accompanied by cyanosis and the vomiting was not frequent. Such a child did not appear to be exhausted; (b) those considered to be moderately ill had frequent paroxysms, moderate cyanosis and frequent vomiting, but not ascertainable serious complications; (c) severely ill infants were those in whom the paroxysms were followed by prolonged prostration and apnea. These last patients often showed signs of extensive pulmonary involvement or symptoms of encephalopathy.

The condition of the patient during the course of treatment with streptomycin was considered to be (a) good when the whoop and emesis were distinctly less within 5 to 7 days after beginning of treatment with streptomycin; (b) unchanged, (c) poor when patient's condition became worse.

Judged from the clinical course of the illness, the intranasal instillation of streptomycin seemed to have no therapeutic value. It should be noted that only three children so treated were severely ill.

from less than 0.5 to 2 μg/ml. Streptomycin nose drops gave negligible levels.

Many bacteria that are sensitive to streptomycin develop a resistance to the drug during therapy. In our experience, whether the streptomycin was given intranasally or by aerosol or intramuscular injection, there was no tendency to develop resistance in the colonies isolated. In all instances, *H. pertussis* remained morphologically unchanged, formed a typical colony, and produced strong hemolysis on Bordet Gengou media. Alexander and Redman (9) report that the emergence of resistance has not been recognized to be a limiting factor in the therapeutic efficacy of streptomycin. Bradford and Day (8) in their experience found that *H. pertussis* sometimes became resistant to streptomycin when a purified powder was sprayed into the nose or mouth.

Comments

Alexander and Redman (7), in an attempt to compare the efficacy of streptomycin and human *H. pertussis* serum, treated thirty infants under 1 year of age, most of whom were less than 6 months old. In all, the infection was proved to be due to *H. pertussis*. The treatment of each new patient was selected on an alternate case basis. Fifteen of this group were treated with streptomycin intramuscularly, 40 μg/kg, eight doses daily, from 4 to 6 days, and an equal number with four vials of human *H. pertussis* hyperimmune serum administered intramuscularly in one dose. The results indicated that the course of the disease had been significantly altered by each agent in the direction of improvement.

Sedallian, Moinecourt, Vialtel, and Del'Hermuzière (10) treated seventeen patients with whooping cough by the intramuscular injection of streptomycin in doses averaging 0.25 gm a day for 4 to 8 days. They believed this was effective in decreasing the number of paroxysms.

Leichenger and Schultz (11) gave 1 gram of streptomycin daily for seven days to eight children by aerosol inhalation. They also gave the same amount for a similar period to eight children by intramuscular injection. Their conclusion was that streptomycin was of clinical benefit in the treatment of whooping cough and stated that the aerosol route of administration was the one of choice.

Conclusions

Streptomycin when administered by aerosol, by intramuscular injection, or by simultaneous administration of both, seemed to have a favorable influence on the course of whooping cough.

There seemed to be no advantage in the combined administration of aerosol and intramuscular injection, when compared with either aerosol or intramuscular injection alone.

When those treated by the intranasal route are excluded, the results are even more favorable. The course of the illness was not so favorable in a group of moderately ill children who did not receive streptomycin.

The course of illness was favorable in two-thirds of the children who were severely ill on admission, and the failures were about 20 per cent (table 58) *There were five deaths in this group, all of whom had also received* human hyperimmune serum.

There seemed to be no advantage in the combined administration of streptomycin by aerosol and intramuscular injection when compared with either aerosol or intramuscular injection alone.

A small number of children who had improved after streptomycin therapy had an increase of paroxysms 2 to 3 weeks later. With one exception, such relapses occurred in children seriously ill on admission.

Complications

It is interesting to note what complications occurred during or after streptomycin therapy. Many younger children were admitted with a pulmonary involvement. In very few, however, was pulmonary involvement first noted during the course of therapy or after its cessation. Upper respiratory infections, which included acute otitis media, were noted infrequently after therapy was discontinued No abnormal neurological manifestations that might be attributed to streptomycin were seen after therapy was stopped.

Nasopharyngeal Cultures

Nasopharyngeal cultures were obtained from nearly every infant on admission. The culture was repeated after the last injection of streptomycin and again just before discharge from the hospital. In the dosage used in the various methods of administration, there was no conclusive evidence that streptomycin had any effect on the presence of *H. pertussis* in the nasopharynx Alexander and Redman (7) reported that following use of streptomycin, they, too, had not been able to demonstrate a significant difference in the speed of disappearance of *H. pertussis* from the nasopharynx. Bradford and Day (8) used a control group and compared it with the streptomycin-treated group. The nasopharyngeal culture for *H. pertussis* by statistical calculation was found to be negative sooner in children who were treated with either aerosol or nasal drops.

Streptomycin Blood Levels and Development of Resistance by H pertussis

Patients receiving streptomycin intramuscularly had significant blood levels, Aerosol-treated patients gave inconsistent blood levels ranging

from less than 0.5 to 2 μg/ml. Streptomycin nose drops gave negligible levels.

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There seemed to be no advantage in the combined administration of aerosol and intramuscular injection, when compared with either aerosol or intramuscular injection alone.

The streptomycin seemed to have a more favorable influence on the course of whooping cough in infants under 1 year of age than in an older group.

The factor of resistance of *H. pertussis* to streptomycin has not yet become a problem in the treatment of whooping cough.

Treatment with streptomycin seemed to have no effect on the presence of *H. pertussis* in the nasopharynx.

It is difficult to compare the effect of various therapeutic agents on the course of an illness, such as whooping cough, which varies widely in different individuals. In our opinion, however, treatment of whooping cough with human hyperimmune serum is more effective than treatment with streptomycin.

Many complications of whooping cough are accompanied by secondary invaders, which are also sensitive to streptomycin. In a seriously ill patient, the most effective treatment at this time is the use of both human hyperimmune serum and streptomycin.

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CHAPTER 28

NONTUBERCULOUS INFECTIONS OF THE BRONCHIAL TREE

Bacterial infections occurring in the trachea and bronchial tree commonly are secondary manifestations of disease. Most instances of chronic bronchitis occur after episodes of acute respiratory disease of viral origin. If, in such cases, bacteria can be isolated, they usually are secondary invaders. Bronchial infection often is a secondary manifestation of some type of pneumonia. Bronchiectasis is primarily a dilation of bronchi; the infection often associated with this disease is a secondary process. The fundamental disturbance in asthma is physiologic; if bacterial infection is present, it generally is a secondary phenomenon and only contributes to the symptom picture.

Primary bacterial infections of the bronchial tree, other than tuberculous lesions, are certainly rare. Because of the motility and contractility of the bronchial tree, because of the action of the cilia in the epithelium of the tree, and especially because of the cough reflex, the bronchi are very efficient in ridding themselves of noxious material. Hence, bronchial infection occurs uncommonly except in association with other disease processes or bronchial obstruction.

BACTERIOLOGIC ASPECTS

The role of antibiotic agents in any infectious process depends upon the bacteriologic character of the infection. Obviously, an antibiotic agent will be of value in the treatment of tracheobronchial conditions only when bacteria are present which are susceptible to that antibiotic agent. The secretions present in association with infections of the tracheobronchial tree usually contain such a multiplicity of bacteria that isolation of significant organisms is difficult. *N. catarrhalis* and *S. viridans* almost always can be recovered from bronchial secretions and these bacteria can be considered as "normal inhabitants." It is doubtful that they are pathogenic. Although both gram-positive and gram-negative bacteria are found in cultures of sputum, gram-positive organisms predominate, as a rule. Hence, penicillin is more commonly indicated in bronchopulmonary sup-

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It is difficult to compare the effect of various therapeutic agents on the course of an illness, such as whooping cough, which varies widely in different individuals. In our opinion, however, treatment of whooping cough with human hyperimmune serum is more effective than treatment with streptomycin.

Many complications of whooping cough are accompanied by secondary invaders, which are also sensitive to streptomycin. In a seriously ill patient, the most effective treatment at this time is the use of both human hyperimmune serum and streptomycin.

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It is not within the scope of this paper to describe the various devices and technical methods for the introduction of streptomycin (or penicillin) into the bronchial tree. Experience has demonstrated that the aerosol method of administering antibiotics is effective in combating the infection and reducing the volume of pulmonary secretions in patients with suppurative bronchiectasis (1, 2). The supraglottic or intratracheal administration of solutions of these antibiotics apparently is a valuable adjunct in some cases (1). At present, inhalation of the dried preparations is being given a trial in the treatment of chronic bronchial infection (11, 12), but as yet it is impossible to predict whether this method will be as effective as the use of aerosol preparations.

The dose of streptomycin employed in inhalation therapy varies from 0.5 to 0.1 gm daily (1, 2). Streptomycin hydrochloride can be mixed readily with penicillin sodium if combined therapy is desired. We have preferred the hydrochloride to the sulfate preparation because the hydrochloride can be readily mixed with penicillin salts, if desired.

At the present writing, experience with dihydrostreptomycin is still insufficient to warrant comment on its value as an agent for aerosol therapy. Results of preliminary studies are encouraging.

Indications for Streptomycin Therapy

Streptomycin is helpful in combating the secondary infection associated with bronchiectasis if the bronchial secretions contain gram-negative, streptomycin-sensitive bacteria (8, 13, 14). In our experience, streptomycin aerosol is indicated for the majority of patients with suppurative bronchiectasis selected for treatment. In many instances the use of streptomycin aerosol makes treatment successful in cases in which penicillin therapy alone has been ineffective. It must be borne in mind that such treatment is purely palliative in bronchiectasis. Aerosol therapy, however, is certainly helping to prepare for operation the candidate for lobectomy, and it helps the patient with bronchiectasis to control bronchorrhea.

Often, patients who have had severe respiratory or influenzal infections will continue to suffer from a productive cough. If culture of sputum is carried out, gram-negative bacteria may be found. Under such circumstances, *H. influenzae* is most commonly demonstrated (8, 14) Bronchial and pulmonary infections caused by *H. influenzae* are frequent and serious in children (13, 14). Streptomycin given either by the intramuscular route or preferably by aerosol will be effective in stopping this cough. It is quite useless, however, to employ antibiotic therapy unless specific bacteria can be isolated.

Obviously, streptomycin can have no effect on the [illegible] disease processes in such conditions as asthma and emphysema. [illegible] bacterial infections associated with these conditions may be

puration than is streptomycin. After penicillin has been used, however, in the treatment of such diseases, culture of bronchial secretions usually reveals pathogenic gram-negative bacteria (1–4). For this reason, streptomycin, either alone or in combination with penicillin, frequently is indicated in the treatment of bronchial infection. The gram-negative bacteria most commonly encountered in bronchiectatic secretions are listed in table 59.

Mode of Administering Streptomycin

The intramuscular method of administering streptomycin has proved to be most advantageous against most of the disease processes in which streptomycin is indicated. All authorities are agreed that tracheobronchial tuberculosis is most effectively treated when streptomycin is given parenterally. The results of treatment of patients with suppurative tracheo-

TABLE 59

Common pathogenic bacteria found in bronchiectatic secretions (17)

GRAM-POSITIVE	GRAM-NEGATIVE
Pneumococcus	*E. coli*
Streptococcus	*H. influenzae*
Hemolytic type	*A. aerogenes*
Nonhemolytic type	*Klebsiella*
Staphylococcus	*Ps. aeruginosa*
Micrococcus	*Proteus*

bronchial disease by intramuscular antibiotic therapy have, however, been disappointing. More satisfactory results have been obtained when penicillin and streptomycin are administered topically. The use of the word "topical" is particularly apt in the case of streptomycin, inasmuch as absorption of streptomycin from the bronchial tree into the blood stream in appreciable quantities does not take place (1, 3–7). In some instances, this is an advantage Because patients with bronchopulmonary suppuration often require treatment for long periods, and because the physician is anxious to avoid the neurotoxic complications of streptomycin therapy, the poor absorption from the bronchial tree actually is desirable.

Several methods have been employed to introduce antibiotic agents into the tracheobronchial tree. Penicillin and streptomycin can be instilled through a bronchoscope (8) or a catheter inserted into the trachea (1). Solutions of antibiotic agents can be instilled by the supraglottic method (1). The patient can inhale these drugs either in aerosol (1–3, 5, 9) or in powdered form. Aerosol preparations usually are made with the aid of nebulizers (1, 3, 5, 9); however, a steam generator can be used for this purpose (10). Devices have been described which permit the inhalation of micronized or powdered penicillin or streptomycin (11, 12).

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CHAPTER 29

LUNG ABSCESS AND EMPYEMA

The treatment of abscess and empyema of the lung is still in its infancy, and it is difficult to indicate with accuracy the result likely to be achieved in any case.

LUNG ABSCESS

Kane and Foley (1) thought that combined penicillin and streptomycin therapy of acute lung abscess had been useful in a single case. Wilson (2) reported little if any improvement in fourteen patients with bronchiectasis or lung abscess following inhalation and intramuscular administration of streptomycin. Army experience with streptomycin in lung abscess has been limited to six cases (3). It would appear that this antibiotic, given intramuscularly in divided dosage of 1 to 2 gm a day, may be of little or no benefit in pulmonary abscess even though the bacteria may be streptomycin-sensitive. Locally instilled streptomycin (1 mg/ml) may, however, be beneficial in openly drained pulmonary abscess caused by susceptible organisms. More experience is needed with streptomycin treatment of acute lung abscess due to penicillin-resistant organisms.

EMPYEMA

Literature review

The literature on the use of streptomycin in empyema is limited. The Committee on Chemotherapeutics and Other Agents of the National Research Council (4) reported that only two of five cases of nontuberculous empyema were improved by streptomycin treatment. Single case reports in the literature are not conclusive (5, 6, 7, 8, 9). In the latest report to the Council on Pharmacy and Chemistry (10), the Streptomycin Committee, Veterans Administration, noted twenty-two of thirty-five cases of tuberculous empyema unimproved.

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Selected cases of acute nontuberculous empyema from streptomycin-sensitive bacteria may be markedly improved by this antibiotic. The

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the whole, our experience with antibiotic aerosol employed alone in the treatment of asthma and emphysema has been disappointing. It is sometimes worth while to combine streptomycin and penicillin aerosol with other accepted methods of treating these conditions (15, 16).

In our experience, toxic reactions to the use of streptomycin by inhalation or by intratracheal instillation have been rare. An occasional patient has had soreness of the mouth and throat. One patient complained of dyspnea. Allergic patients are most likely to have a limited tolerance for streptomycin aerosol. No vestibular disturbances have been encountered among patients treated with aerosol streptomycin.

The phenomenon of resistance of bacteria to streptomycin develops when aerosol preparations of streptomycin are used, just as it occurs when streptomycin is administered intramuscularly. This fact puts a definite limitation on the value of streptomycin aerosol in the long-term treatment of suppurative bronchiectasis. Resistant bacteria have developed in the bronchial secretions of a number of our patients, and additional inhalation treatment has been ineffective. *E. coli* is particularly likely to become a resistant organism. Yet, despite the resistance factor, it has been our opinion that streptomycin is a valuable drug in inhalation therapy.

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CHAPTER 29

LUNG ABSCESS AND EMPYEMA

The treatment of abscess and empyema of the lung is still in its infancy, and it is difficult to indicate with accuracy the result likely to be achieved in any case.

LUNG ABSCESS

Kane and Foley (1) thought that combined penicillin and streptomycin therapy of acute lung abscess had been useful in a single case. Wilson (2) reported little if any improvement in fourteen patients with bronchiectasis or lung abscess following inhalation and intramuscular administration of streptomycin. Army experience with streptomycin in lung abscess has been limited to six cases (3). It would appear that this antibiotic, given intramuscularly in divided dosage of 1 to 2 gm a day, may be of little or no benefit in pulmonary abscess even though the bacteria may be streptomycin-sensitive. Locally instilled streptomycin (1 mg/ml) may, however, be beneficial in openly drained pulmonary abscess caused by susceptible organisms. More experience is needed with streptomycin treatment of acute lung abscess due to penicillin-resistant organisms.

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divided daily dosage should amount to 2 to 3 gm intramuscularly and 1 or 2 gm locally either into the closed or open cavity. Following is a case in which streptomycin was probably life-saving:

A 26-year-old male, following a nontraumatic perforation of the sigmoid colon, successively developed peritonitis, a right-sided diaphragmatic abscess, and a severe right-sided empyema. Shortly after drainage of the subdiaphragmatic abscess, massive collapse of the right lung occurred. The lung re-expanded partly, but the patient's condition became critical Culture of pus from the chest, 150 cc of which was removed by thoracentesis, revealed *E. coli* as the predominating organism. Streptomycin therapy was therefore begun, in the amount of 2 4 gm daily by the intramuscular route and 2 0 gm daily by the intrapleural route after aspiration. The result was prompt and gratifying Temperature, pulse, and respiration fell to normal levels, there was no further weight loss and the patient's appetite improved. By the end of 26 days of treatment the patient's condition was sufficiently stabilized to permit right thoracotomy, with drainage of the residual empyema. Streptomycin was continued by the intramuscular route for 8 days after operation; at the end of this time the temperature was normal The patient remained permanently afebrile, and the empyema cavity ultimately was obliterated.

The next case emphasizes the unwisdom of attempting to manage a loculated empyema cavity without surgical drainage:

An 18-year-old soldier with unresolved pneumonia of the left lower lobe on a bronchiectatic basis developed an acute sacculated empyema of the left pleural cavity with a pronounced constitutional reaction Aspiration yielded thick greenish yellow pus from which streptomycin-sensitive *H. influenzae* and anaerobic nonhemolytic streptococci were grown Penicillin and sulfadiazine were both ineffective. Streptomycin (0 5 gm) was then given intramuscularly every 4 hours for 10 days Simultaneously, an attempt was made daily or every second day to empty the various loculated empyema cavities and to replace the aspirate with 0 25 gm of streptomycin in solution For 3 days after this treatment was begun, the temperature was lower and the patient appreciably improved Then drainage of the pockets by aspiration became increasingly difficult, and the temperature returned to its former high peaks Eventually the cavity was completely obliterated by thoracotomy and open drainage, under cover of parenteral penicillin and streptomycin. Three months later a plastic operation on the chest wall was carried out, and the soldier was in good condition when he was subsequently separated from the service

Radical and curative surgery may be made possible by judicious local application of streptomycin. Systemic use of the antibiotic may not be effective alone, since bacteriostatic concentrations by way of the blood stream are not achieved in fibrotic poorly vascularized tissues In the following case, furthermore, the indications for streptomycin therapy were clean-cut because the staphylococci had become penicillin-fast:

A 35-year-old man, first seen in June 1947, had a persistent empyema as the result of a shell fragment in 1944 Thoracotomy and several thoracoplasties had been ineffective In July 1946, decortication of the empyema space was followed by a break-

down of the wound, which resulted in a saucerised cavity and the exposure of a large area of visceral pleura. Hemolytic *S. aureus* infection was persistent, and the course was septic. A split-thickness cutaneous graft did not take. In July 1947, culture of contents of the empyema cavity again yielded hemolytic *S. aureus*, which was now resistant to penicillin. The bacterium, however, was sensitive to streptomycin, which was given in dosages of 2 gm daily intramuscularly. The temperature almost immediately returned to normal, and the amount of drainage decreased. The cavity was then packed with gauze saturated with 1 gm of streptomycin in isotonic NaCl, and several sterile samples were obtained over a 10-day period. It was then possible to decorticate the visceral pleura, to perform a plastic operation on the thoracic muscles, and to close the chest wall. The wound healed promptly and firmly, and the patient was in good health when he left the hospital after operation.

In *mixed tuberculous empyema* streptomycin given intramuscularly in divided dosage of 2 gm daily and also intrapleurally, especially when given with penicillin, can suppress the pyogenic bacteria. Although streptomycin appears to be of no value in the parenchymal lesion of chronic active tuberculosis with empyema, it may allow operative attack on the visceral fibroplastic membranes and also extensive plastic procedures on the chest without incurring spread of the tuberculous infection. Following is an exemplary case:

A 48-year-old Negro man with moderately advanced tuberculosis of the right lung developed a mixed tuberculous empyema of the right pleural cavity. Examinations of the sputum were consistently negative for acid-fast bacilli, but cultures from the empyema cavity were positive for these bacilli on several occasions and were also positive for hemolytic *S. aureus*. After the cavity had been unroofed and exteriorized, roentgenograms showed another large pocket in the right hemothorax, the parietal pleura was greatly thickened, and the right lung was compressed to about a quarter of its normal volume. Penicillin by the parenteral route and azochloramide used locally were not effective; but over the next 4 months, under the influence of bed rest and supportive therapy, the temperature gradually fell to 99°F. Thoracoplasty, followed by two revisions, failed to eradicate the infection or close the cavity. Two months later, when the patient began to expectorate rather large amounts of frothy sputum, repeated examinations of the sputum were still negative for acid-fast bacilli. Streptomycin therapy was instituted at this time, and 2 gm was given daily for 185 days. The patient's cough decreased and became less productive; drainage from the empyema stoma became negligible, the general condition of the patient improved, he began to gain weight, and the residual fistula eventually closed. The final radiograph showed the left lung clear, and on the right there was complete compression of the entire lung field, without evidence of empyema.

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CHAPTER 30

URINARY TRACT INFECTIONS

The earliest reports indicated that streptomycin effectively inhibited the growth of most of the gram-negative bacteria responsible for infection of the urinary tract. Clinical studies have defined the importance of method of treatment, anatomical conditions prevailing in the genito-urinary tract, and the bacterial flora producing infections as factors influencing the results obtained from the treatment with streptomycin of various types of genito-urinary infections. This chapter reviews briefly the basic pharmacologic and bacteriologic data of direct importance in the treatment of these infections with streptomycin and summarizes the use of this chemotherapeutic agent in their management.

PHARMACOLOGY

Streptomycin administered intramuscularly produces maximum blood levels in 2 to 3 hours after injection and appears rapidly in the urine during the first hour after administration. Although the urinary excretion of the drug is greatest during the 4 hours after administration, excretion continues at a considerably slower rate than that of penicillin. Whereas 1 hour after injection about 60 per cent of a given dose of penicillin can be recovered from the urine and excretion is almost complete within 4 hours, 20 to 30 per cent of a single dose of streptomycin is excreted later than 4 hours after administration.

The concentration and the total amount of streptomycin excreted in the urine are variable and are related to the dose of the drug, urine volume, and renal function. In our cases in which it has been feasible to limit the daily fluid intake to approximately 2,500 ml and with a daily dose of 1 gm of streptomycin, a minimum urinary concentration of 75 to 100 μg/ml has been present almost uniformly. Two grams of streptomycin daily usually insures a urinary concentration of 250 μg/ml. Administration of oral alkali has produced no significant effect on the urinary excretion of streptomycin. Occasional instances may be observed of patients with advanced renal

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mycin are discussed in detail in other chapters. Most of the bacteria responsible for urinary tract infections are sensitive to the concentration of streptomycin readily obtainable in the urine, although naturally resistant cultures are occasionally encountered, especially with *Ps. aeruginosa* and *S. faecalis*. Although gram-negative bacilli are usually sensitive *in vitro*, Helmholz (1) found that when urine was employed as a culture medium a concentration of less than 66 μg/ml produced no interference with growth, and in the case of *Ps. aeruginosa* or *S. faecalis* a concentration of at least 100 μg/ml was necessary to produce a bactericidal effect. A wide margin of safety is, therefore, probably important in determining dosage in relation to the *in vitro* sensitivity. This is further emphasized by the knowledge that the number of bacteria in infected urine usually greatly exceeds the size of the inoculum employed for the *in vitro* sensitivity test. Inasmuch as the magnitude of the initial bacterial population is of great importance in the appearance of streptomycin-resistant bacteria, the large number of organisms in urinary tract infections may be partly responsible for the readiness with which this phenomenon occurs. Bacteria recovered from patients with urinary tract infections which are not sterilized with streptomycin usually grow readily in the presence of concentrations of 25,000 to 50,000 μg/ml This represents absolute resistance for therapeutic purposes, although the sensitivity of these bacteria to other chemotherapeutic agents is not altered. The rapidity with which resistant bacteria emerge during treatment of urinary tract infections is striking. The bacterial population of the urine may have become highly resistant 12 hours after initiation of streptomycin therapy, and in our experience this phenomenon has become manifest without exception after 72 hours of treatment if bacilluria continues.

Clinical Features

Acute pyelonephritis

Acute urinary tract infections accompanied by marked constitutional symptoms and signs manifest dramatic clinical improvement in at least 75 per cent of cases Rapid decrease of fever, costovertebral angle tenderness, leukocytosis and pyuria, and improvement in renal function occur. Bacteremia coexists in at least 15 per cent of cases of acute pyelonephritis, the presence of which is an index of the severity of infection, since many of these cases represent the most serious of the gram-negative bacillary infections. The grave prognostic significance of this finding has been emphasized previously (2), but in addition bacteremia is usually found in patients with serious underlying disease which in itself may be of great importance in determining the results of treatment Control of bacteremia may be an important factor in producing a good clinical response; the effectiveness

disease who manifest decreased renal excretion of streptomycin, permitting the accumulation of high serum streptomycin concentrations and urine levels which may be inadequate to produce bactericidal effects.

Bacteriology

The bacteria isolated most frequently from urinary tract infections are gram-negative bacilli of which *E. coli* and *A. aerogenes* predominate. Gram-positive cocci may be recovered in either pure or mixed culture in about 25 per cent of infections. Staphylococci are most common. Among the streptococci the nonhemolytic enterococcus group and alpha-hemolytic cocci occur more frequently than beta-hemolytic types. Table 60 illustrates the relative frequency of various gram-negative bacteria in pure in-

TABLE 60

Occurrence of gram-negative bacilli in urinary tract infections (3)

		NUMBER OF CASES	PERCENTAGE OF LOSSES
E coli		513	45
A aerogenes		116	10
Ps aeruginosa		135	12
Pr vulgaris		79	7
Kl pneumoniae		14	1
H influenzae		1	
Mixed infections		294	25
Mixed gram-negative	140		
Mixed gram-negative and gram-positive	154		
Total		1,152	100

fections and indicates the occurrence of mixed infections with several types of bacteria The frequency with which groups of bacteria different from those originally isolated appear during treatment suggests that mixed infections may be more frequent than is superfically evident and that infections due apparently to a single type of organism may represent only a predominance dependent on conditions prevailing at the particular time the urine was cultured Change of the bacterial flora has been observed in about 25 per cent of our cases Catheterization or urological procedures may frequently be responsible for such a phenomenon Thus, gram-positive cocci resistant to streptomycin may appear in the urine after disappearance of the initial gram-negative population. The appearance of actual infections with gram-positive cocci during or following streptomycin therapy has been emphasized elsewhere.

The sensitivity of bacteria and the development of resistance to strepto-

ished and may be appreciable even following bacteriologic cure. Pyuria has disappeared in only about 30 per cent of our own cases in which urinary sterilization has occurred. The significance of this finding and its relation to relapse is not entirely clear.

The results of treatment differ markedly, depending on the presence of anatomical obstruction to the free flow of urine, advanced pathological change in the urinary tract, and the presence of foreign bodies such as calculi. In one series (4) urinary sterilization occurred in 17 per cent of the patients with obstructive uropathy, compared with 79 per cent of those in which no obstructive element could be demonstrated. In another (3), 784 patients with chronic urinary tract infections were treated with streptomycin. Obstructive uropathy was the most frequent coexistent disease and

[illegible]

tion in 47 per cent, compared with permanent clinical improvement in 72 per cent and disappearance of bacilluria in 65 per cent of the group without obstructive uropathy or other coexistent disease. Similarly, nephrolithiasis coexisted in 96 patients, of whom 59 per cent manifested clinical improvement and 52 per cent urinary sterilization. In our own cases and those of others good clinical results and disappearance of bacilluria have been noted even more infrequently, although clinical improvement occurs particularly when acute constitutional symptoms are present. Most of the cases of nephrolithiasis in which good bacteriologic results have been obtained have undergone nephrectomy, thus making it impossible to ascribe bacteriologic cure to streptomycin. The rare cases showing urinary sterilization in the presence of calculi in our own experience have relapsed shortly after cessation of chemotherapy. In general, the factors influencing results in chronic urinary tract infections are similar to those in acute infections, although more cases may be seen in which failure can be ascribed to no definite cause other than urinary tract disease of longstanding, the significance of which has been previously shown in connection with other chemotherapeutic agents

The frequent appearance of mild albuminuria and casts and the occasional occurence of nitrogen retention following institution of streptomycin therapy are well recognized. Certain patients with chronic renal disease and urinary tract infection may manifest an apparent acceleration of the clinical picture of renal failure with increasing azotemia following administration of streptomycin. Acute episodes of gout accompanied by increased serum uric acid have been observed in two such cases presenting a previous history of gout. A marked hypotensive effect has been noted following chemotherapy in several patients with chronic renal disease and hypertension, although both increasing azotemia and decreased blood pressure must

with which streptomycin controls bacteremia, and the more dramatic response of acute constitutional symptoms, may be responsible for the slightly better clinical results observed in acute as compared with chronic infections. Urinary sterilization has been observed less frequently in patients with bacteremia. Bacilluria disappeared in 43 per cent of 62 patients with bacteremia and in 60 per cent of 402 patients without bacteremia. In the cases studied by Keefer and Hewitt (3) under the auspices of the National Research Council, bacteriologic cure was obtained in 58 per cent of the 464 acute infections and with the same frequency in chronic infections. In our own cases (4) such a high cure rate has been obtainable only with the concomitant use of alkali, and the incidence of bacteriologic cures in the immediate sense has been somewhat less in acute than in chronic types of infection although relapse is notoriously frequent in the latter. Failure of the urine to become sterile before complete clinical recovery in acute pyelonephritis has also been noted frequently with sulfonamide therapy (2). Even if urinary sterilization occurs, pyuria may continue and presage relapse of bacteriuria. In spite of failure to accomplish bacteriologic cure the clinical improvement which occurs so regularly in acute pyelonephritis may be in itself of considerable value in the management of acute exacerbations of upper urinary tract disease in patients with neurologic bladders or on catheter drainage or in preparation of a patient for definitive treatment by surgery.

Pyelonephritis of pregnancy

Cases of pyelonephritis of pregnancy with moderate or severe constitutional symptoms and signs rather than merely pyuria or bacilluria manifest an excellent clinical response with decreased bacilluria and pyuria in almost all cases. The incidence of bacteriologic cure corresponds closely with that obtained in other acute types of infection. Inasmuch as symptoms and fever commonly subside on a regimen of bed rest and administration of large volumes of fluid, evaluation of the role played by streptomycin in these cases is somewhat difficult. Bacteriologic relapse is frequent in these patients, usually appearing within 1 month following termination of chemotherapy. The most notable response of pyuria occurs in this group of cases, especially if urinary sterilization occurs.

Chronic pyelonephritis

The response of the mild urinary tract symptoms and low grade fever associated with chronic pyelonephritis has been irregular in contrast to the dramatic response of acute pyelonephritis. Bacilluria usually responds better than pyuria The former, if not eradicated, is often considerably decreased at least temporarily, but the latter frequently continues undimin-

tion on high daily doses (4). Highly resistant bacteria invariably appear. Use of streptomycin in the presence of catheter drainage should be confined to patients with severe, uncontrolled upper urinary tract disease or bacteremia associated with disease of the urinary tract. The treatment of lower urinary tract infections with persistent dysuria and pyuria in the immediate postoperative period following transurethral prostatic resection has also been most disappointing. Streptomycin offers no solution to this common and troublesome problem, and its use is followed almost invariably in our experience by the appearance of resistant bacteria with no permanent symptomatic improvement.

Urinary tract infections associated with paraplegia

The treatment of many paraplegic patients with urinary tract infections is complicated by the necessity for catheter drainage. Use of streptomycin in such patients should be confined, as in other patients with catheter drainage, to instances of acute upper urinary tract disease with or without bacteremia In patients with automatic bladders and no residual urine, good clinical results with urinary sterilization have been reported (5). Reinfection will occur in many instances especially if residual urine is present. In the series of 241 cases reported by DeBakey and Pulaski (5), improvement was noted in 35 per cent of patients in whom calculi were absent Improvement occurred in 11 per cent of patients with calculi and in only 7 per cent of those in whom undrained abscesses or cellulitis was present.

Acute epididymitis

The incidence of acute epididymitis following lower urinary tract operative procedures has been greatly decreased by preliminary vasectomy. About 50 per cent of our own and of the reported cases respond dramatically to streptomycin, with rapid decrease in pain, tenderness, and scrotal swelling, obviating the necessity for further surgery. Bacilluria has continued in all our own cases.

Cystitis

The dysuria and frequency associated with acute cystitis have shown a dramatic response to streptomycin in almost all cases, and urinary sterilization has been noted slightly more frequently than in patients with evidence of predominantly upper urinary tract disease, although the frequency of renal infections associated with cystitis is well recognized The use of streptomycin in patients presenting urgency, frequency, suprapubic pain, and markedly diminished bladder capacity with relief of symptoms following urination and without evidence of bacterial infection, that is, the clinical picture of interstitial cystitis, has been without effect in our experience

be characterized as occasional rather than regular phenomena in this class of patient.

Preoperative and postoperative use of streptomycin in relation to genito-urinary surgery

The value of streptomycin in controlling acute constitutional symptoms frequently with bacteremia and in improving renal function, thus permitting genito-urinary surgery under considerably more auspicious circumstances, has been mentioned previously. Certain situations exist in which sulfonamides are contraindicated and streptomycin is the agent of choice, as in ureteral obstruction with stone, stricture, or tumor, in the presence of markedly impaired renal function, in the elderly where difficulties of fluid intake and nutrition constitute relative contraindications, and when sulfonamides have previously failed or evidence of sensitization is present. Resistant bacteria may arise when streptomycin is employed preoperatively under circumstances which, because of the presence of obstructive uropathy, undrained abscesses, calculi, or catheters, are unfavorable for bacteriologic cure. Persistence of these resistant bacteria may preclude the successful use of streptomycin in the postoperative period, and streptomycin-resistant bacteremia may arise from the urinary tract focus, thus emphasizing the importance of reserving streptomycin, insofar as possible, for a time when circumstances for cure are most favorable When the main source of infection is to be removed, as in nephrectomy, this difficulty may be obviated to some extent Although temporary improvement may occur following streptomycin therapy of patients with established perinephric or renal abscesses, permanent improvement is rare indeed unless chemotherapy is combined with surgical drainage. In cases in which bacilluria has been present preoperatively, urinary sterilization occurs relatively infrequently even with the latter type of management. Disappearance of bacilluria has occurred in about 20 per cent of our own cases and of these the majority have undergone nephrectomy, thus bringing into question the part that streptomycin has played in elimination of infection. Streptomycin, as well as other chemotherapeutic agents, has been uniformly unsuccessful in sterilizing the urine in the presence of catheter drainage with indwelling urethral, suprapubic, or nephrostomy tubes, which act as foreign bodies preventing eradication of infection and serve as a convenient portal of entry for reinfection. The frequency with which infection appears or the bacterial flora of the urine changes after catheterization emphasizes the importance of the procedure and the careful technic which should be employed. The inefficacy of streptomycin under these conditions is illustrated by the inability of the drug to maintain the urine sterile even when patients with no urinary infection are started prior to and maintained after catheteriza-

Relapses

Accurate knowledge of the relapse rate of gram-negative bacillary infections of the urinary tract following streptomycin or other chemotherapy is not available beyond the general knowledge that recurrence of bacilluria is very frequent. The patients studied in cooperation with the National Research Council (3) showed a relapse rate of 9.4 per cent, probably much below the true figure, because of the short or inadequate follow-up characteristic of such a study. The relapse rate in our own cases has been almost 25 per cent within 6 months after treatment. If one excludes the cases of pyelonephritis of pregnancy and urinary tract infections following catheterization or urologic study procedures, which represent usually only transient episodes that receive early and adequate treatment, the frequency of relapse is even more striking. The data obtained from the National Research Council series, however, indicate the time at which relapse is observed. In patients in whom recurrence of bacilluria occurred, it was noted in 57 per cent within 1 week of termination of chemotherapy, between 1 and 4 weeks in 27 per cent, and later than 1 month after treatment was discontinued in 16 per cent.

Method of Treatment

Time-dosage relationships are of great practical importance in the management of patients. Streptomycin has appreciable toxicity, in contrast to penicillin, and it is therefore important to keep the dosage as low as is commensurate with obtaining the best result. Determination of bacterial sensitivity is of only occasional practical importance, since streptomycin is bactericidal for the majority of gram-negative bacilli encountered at present, with the exceptions noted below, and the urinary concentration with the recommended dosage usually far exceeds the *in vitro* sensitivity. In the presence of bacteremia this determination is of importance as a guide to dosage, inasmuch as the serum concentration should at least exceed, preferably by several times, the *in vitro* sensitivity.

The correlation of average daily dosage with clinical and bacteriologic result obtained in a large series of patients (3) is presented in table 61. The clinical results with a daily dosage of 1 gm were as good as those with higher dosage, and the bacteriologic result following a dose of 1 gm did not differ significantly from those receiving higher dosage. The data of numerous smaller series are in accord with this finding, and the conclusion would seem justified that 1 gm daily is a dose sufficient to achieve the best clinical and bacteriologic result. With this dose a urine concentration of streptomycin less than 100 μg/ml will be observed only occasionally, provided the fluid intake is limited to 2,500 ml a day. This represents a useful method for conserving material, decreasing the incidence of toxic reactions, and in-

Cases characterized by acute hemorrhagic cystitis from which *E. coli* is commonly isolated have responded well to streptomycin. The usual course of this disease, however, is relatively short and self-limited, the response to instillations of argyrol good, and the part that either local or systemic treatment plays in modification of the natural cause of the disease difficult to evaluate. Dienes, Ropes, Smith, Madoff, and Bauer (6), have noted a satisfactory response to chemotherapy in similar cases from which pleuropneumonia-like organisms were isolated.

Nongonococcal urethritis and prostatitis[2]

Inflammation of the urethra and prostate due to gram-negative bacilli and to penicillin-resistant gram-positive cocci is not infrequent. The information regarding the usefulness of streptomycin in these cases is relatively limited. Cases of urethritis that clinically do not present prostatic involvement have usually responded well. The results are poorer when prostatitis exists, the symptoms and urethral discharge subsiding usually only temporarily, if at all, or recurring promptly after cessation of chemotherapy.

Cases of purulent urethritis occur, sometimes accompanied by prostatitis and cystitis that do not present a clear bacterial etiology. Urethritis may be associated in some instances with arthritis and conjunctivitis to comprise Reiter's syndrome. Pleuropneumonia-like organisms have been isolated from some of these cases, which fail to respond to sulfonamides or penicillin, particularly if there has been previous treatment with the latter agent. Rapid improvement in symptoms, subsidence of urethral discharge, and disappearance of pleuropneumonia-like organisms from urethral cultures have been noted following streptomycin therapy in some patients who present genito-urinary involvement alone. Dienes has pointed out the difficulty in evaluating the effectiveness of streptomycin in cases of nonspecific genito-urinary disease from which pleuropneumonia-like organisms have been isolated, since the natural course of the disease is frequently a self-limited one with a tendency to spontaneous subsidence of symptoms and disappearance of pleuropneumonia-like forms over a relatively short period. The value of streptomycin is considerably clearer in patients with disease of long duration, in whom a striking effect occasionally may be observed following chemotherapy. Some cases of Reiter's syndrome have shown rapid improvement of genito-urinary tract symptoms and signs but more gradual improvement during and after streptomycin therapy with respect to symptoms related to the arthritic component of the syndrome. Relapses have been observed in both types of cases (6, 7).

[2] The suggestions and criticism of Dr Howard Weinberger are gratefully acknowledged.

successfully treated with a relatively low total dosage. The correlation between clinical and bacteriologic results and total dosage is presented in table 62. There is no relation between clinical result and total dosage, and the similarity of the bacteriologic results obtained with varying total dosage is quite striking. One may reasonably conclude that large total doses have little place in the treatment of urinary tract infection.

Because the rate of excretion of streptomycin is considerably slower than than that of penicillin, injections may be spaced at longer intervals. The data in table 63 justify the conclusion that injections at 6-hour intervals are adequate to obtain optimum results, and there is suggestive evidence regarding injections spaced at even wider intervals (3).

Inasmuch as streptomycin represents not the initial agent but rather an

TABLE 62

Results on urinary tract infections correlated with total dosage of streptomycin (3)

TOTAL DOSAGE	NUMBER OF CASES	PERMANENT IMPROVEMENT		TEMPORARY IMPROVEMENT OR NO EFFECT		URINE CULTURE AFTER TREATMENT			
						Sterile		Positive	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
5 gm or less	574	414	72	160	28	337	59	237	47
6–20 gm .	622	419	67	203	33	362	58	260	42
Over 20 gm .	51	28	55	23	45	27	53	24	47
Not specified	1	1	100	—	—	1	100	—	—
Totals	1,248	862		386		727		521	
Percentages			69		31		58		42

addition to the armamentarium available for the treatment of urinary tract infections, the effect of concomitant treatment with this agent and other available preparations is of interest. The evidence is inadequate to justify any conclusions in this regard, although the demonstration by Kolmer of the effectiveness of combined treatment with streptomycin and sulfadiazine in experimental *Kl. pneumoniae* infections suggests the use of both agents in these infections (8) Patients treated surgically in conjunction with chemotherapy for the drainage of abscesses, relief of urinary obstruction, or removal of calculi generally do very well. In the National Research Council series (3) 87 per cent of the patients treated with both surgical and chemotherapeutic measures manifested clinical improvement, as compared with 69 per cent in the entire group. This is particularly notable in view of the advanced character of the pathological process present in these patients Sterilization of the urine is less frequent than the good

creasing the concentration of streptomycin in the urine. When fluid intake must be maintained at a higher level, the dose of streptomycin should be correspondingly increased. The type of bacteria responsible for a given infection may be a factor in determining dosage. When bacteria of known borderline resistance are present or when *Ps. aeruginosa* or *S. faecalis* is cultured from the urine a minimum dosage of 2 gm is advisable. This procedure has produced better results in our own cases, although its effectiveness was not confirmed in the above series, in which no difference was observed between these doses. The presence of bacteremia also justifies higher dosage, and at least 2 gm daily is recommended. Smaller doses should be used for infants and children; 25 mg per pound of body weight is

TABLE 61

Results on urinary tract infections correlated with average daily dosage of streptomycin

DAILY DOSAGE	NUMBER OF CASES	PERMANENT IMPROVEMENT		TEMPORARY IMPROVEMENT OR NO EFFECT		URINE CULTURE AFTER TREATMENT			
						Sterile		Positive	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Less than 1 gm	132	90	68	42	32	78	59	54	41
1 gm	673	493	73	180	27	398	59	275	41
2 gm	359	224	62	135	38	196	55	163	45
More than 2 gm	84	55	65	29	35	55	65	29	35
Totals	1,248	862		386		727		521	
Percentages			69		31		58		42

quite adequate even in the presence of bacteremia, and 0.25–0.5 gm daily is sufficient for uncomplicated infections.

Treatment for 5 to 7 days usually suffices to obtain the best clinical results Occasionally, however, clinical improvement may continue with gradually decreasing fever and pyuria, in the presence of which continued treatment is quite justifiable. Likewise, clinical improvement may continue in spite of failure to sterilize the urine. The bacteriologic results were as good in patients treated for less than 5 days as in the other groups. This is explained by the appearance of urinary sterilization, if it is to occur, within 3 days of initiation of chemotherapy and usually within a shorter period of time. There have been no exceptions to this in our own experience. The presence of resistant bacteria after this period of treatment precludes any improvement in bacteriologic result.

A corollary to the recommendations regarding average daily dosage and duration of treatment should be that most urinary tract infections may be

treatment of urinary tract infection. In defining the results of chemotherapy of these infections, one should remember the importance of long-term follow-up and bear in mind that the results so far obtained are relatively immediate in point of time. Data are not yet available concerning the permanency of the cures obtained, and it has been shown that the usual laboratory tests are of little value in prognosis for patients in whom urinary tract infections have been arrested with sulfonamides (2).

Age

Table 64 presents the correlation between age and results of treatment of the patients studied during the National Research Council program (3). The clinical results were essentially the same in all age groups, but the frequency of bacteriologic cure is significantly less in patients more than 40 years old.

TABLE 64

Results of streptomycin on urinary tract infections correlated with age of patients

AGE	NUMBER OF CASES	PERMANENT IMPROVEMENT		TEMPORARY IMPROVEMENT OR NO EFFECT		URINE CULTURE AFTER TREATMENT			
						Sterile		Positive	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
0–20 years	164	118	72	46	28	102	62	62	38
21–40 years	324	214	66	110	34	210	65	114	35
41–60 years	420	293	70	127	30	232	55	188	45
Over 60 years	327	229	70	98	30	177	54	150	46
Not specified	13	8	62	5	38	6	46	7	54
Totals	1,248	862		386		727		521	
Percentages			69		31		58		42

Sex

The correlation between sex and results of treatment is similarly shown in table 65. The clinical results were slightly but significantly better in females than in males, and the bacteriologic results were considerably better in the females. This was true for patients in the older as well as in the younger groups.

Duration of prior illness

The importance is well established of duration of prior illness in producing the chain of events associated with chronic pyelonephritis In the cases treated under the National Research Council program (3) only a

clinical results might indicate. This would follow logically from the necessity for frequent use of catheters to assure the free flow of urine and the presence following urinary tract surgery of wounds with granulating surfaces which offer very favorable conditions for bacterial growth.

Strikingly improved results are obtained by the concomitant use of streptomycin and alkali in dosage sufficient to maintain the urine at an alkaline pH at which the activity of streptomycin is considerably enhanced. Oral administration of sodium bicarbonate or of sodium or potassium citrate in doses of 2 gm every 4 hours usually suffices to maintain the urine alkaline. Potassium citrate (50 per cent solution) is employed in patients with cardiac disease. Objections may be raised to the use of concomitant alkali when *Pr. vulgaris* is present in the urine, since an alkaline medium favors an increased rate of growth of this organism and precipitation of calcium

TABLE 63

Results of streptomycin on urinary tract infections correlated with interval between injections of streptomycin

INTERVAL	NUMBER OF CASES	PERMANENT IMPROVEMENT		TEMPORARY IMPROVEMENT OR NO EFFECT		URINE CULTURE AFTER TREATMENT			
						Sterile		Positive	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
3–4 hours	1,119	777	69	342	31	650	58	469	42
6 hours	102	65	64	37	36	60	59	42	41
Not specified	27	20	74	7	26	17	63	10	37
Totals	1,248	862		386		727		521	
Percentages			69		31		58		42

phosphate In pure *Pr. vulgaris* infections the use of alkali may be unnecessary, since the urine is usually already alkaline, but in mixed infections the urinary pH should be followed carefully because with the eradication of *Pr. vulgaris* the urine may become acid and alkali therapy may be required. It should be borne in mind that complications incident to fluid retention may arise following alkali therapy in cardiac patients and those with azotemia

The local use of streptomycin in the bladder or renal pelves by irrigation through catheters has not been beneficial in the small number of reported cases.

Factors Determining Results

A preliminary classification may be made concerning the importance to therapeutic success of the various factors that must be considered in the

may be due to the urea-splitting ability of this organism with the formation of an alkaline urine, in which streptomycin is more active. It will be occasionally observed that in mixed infections with *Proteus* this organism will disappear, the urine will become acid, and other members of the bacterial flora may persist. The presence of *Proteus* should suggest careful search for calculus formation, which influences adversely the results obtained with all chemotherapeutic agents. Some cultures of *Ps. aeruginosa*, *S aureus*

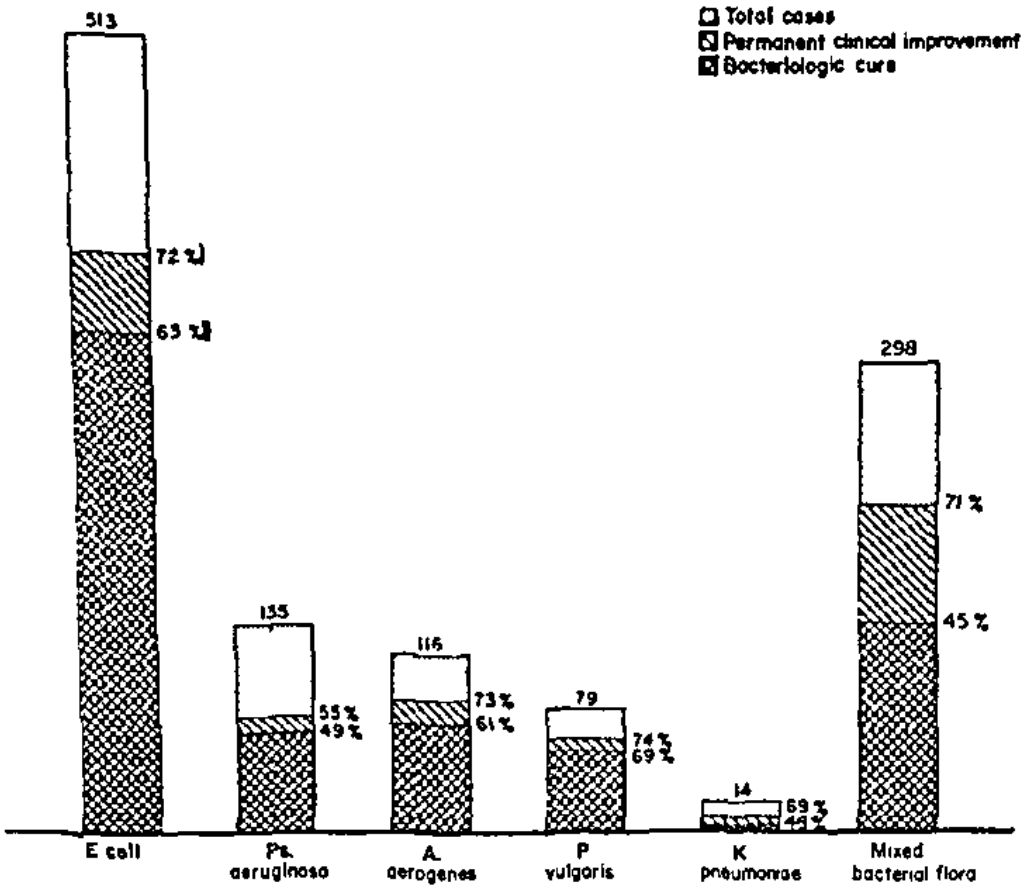

FIG 71 Relation of bacterial flora to results of treatment with streptomycin in urinary tract infections.

and *albus*, and *A. faecalis* also may split urea and produce ammonia and a strongly alkaline urine conducive to stone formation.

The clinical results are poorer and sterilization of the urine is less frequent in the presence of mixed infections than in the presence of a single type of bacteria. This relationship is shown in figure 71. The anatomical lesions associated with mixed infections are also frequently more complex.

Use of alkali

Strikingly improved results are obtained by the concomitant administration of streptomycin and alkali in sufficient dosage to maintain the urine at an alkaline pH, at which the activity of streptomycin is considerably

slight tendency was noted toward poorer clinical and bacteriologic results with increasing duration of illness, and this tendency was more marked in urinary tract infections accompanied by acute constitutional symptoms and signs than in chronic types of infection. In our own cases the importance of duration of illness has been more prominent in determining both immediate response and frequency of relapse. The effect of duration of illness is frequently difficult to evaluate because of the irregular course and response of low-grade urinary tract symptoms and low-grade fever associated with chronic pyelonephritis.

Bacterial flora

Within the range of bacterial sensitivity to streptomycin which governs the treatment of urinary tract infections, variation in sensitivity does not

TABLE 65

Results of streptomycin on urinary tract infections correlated with sex of patients (3)

SEX	NUMBER OF CASES	PERMANENT IMPROVEMENT		TEMPORARY IMPROVEMENT OR NO EFFECT		URINE CULTURE AFTER TREATMENT			
						Sterile		Positive	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Male	660	433	66	227	34	330	50	330	50
Female	581	423	73	158	27	392	67	189	33
Not specified	7	6	86	1	14	5	71	2	29
Totals	1,248	862		386		727		521	
Percentages			69		31		58		42

appear to be of great importance. Harrell, Herndon, Gilliken, and Aikawa (9) have shown that bacteria sensitive to a concentration of 2 μg/ml before treatment were as likely to be replaced by highly resistant bacteria of the same type as were organisms with an initial sensitivity of 32 μg/ml. If the sensitivity of the bacteria present is within this range, little benefit appears to be derived by increasing the daily dosage beyond 1 gm.

Ps. aeruginosa and *S. faecalis* both occur frequently in urinary tract infections and are usually more resistant to streptomycin than other commonly occurring bacteria. The results have been correspondingly poor when these bacteria are the etiologic agent. More extensive urinary tract pathology has been said to accompany infection with *Ps. aeruginosa*, as well as with *A. aerogenes*, although the evidence for this is not entirely clear. Infections with *Pr. vulgaris*, on the other hand, have shown urinary sterilization more frequently than infections with other bacteria. This

with the urinary tract offer such favorable conditions for bacteriological growth that sterilization of the urine cannot be accomplished under such conditions. Under these circumstances reduction of the number of bacteria in the urine may occur with streptomycin therapy, but the bacterial count returns to its former level either during or immediately after termination of treatment, and the usefulness of streptomycin is confined for the most part to control of acute upper urinary tract disease with acute constitutional symptoms and signs.

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increased. In our cases treated without alkali, urinary sterilization has occurred in 20 per cent, whereas disappearance of bacilluria has resulted in 73 per cent of those patients given concomitant alkali. Administration of alkali has not influenced the appearance of resistant bacteria in cases with persistent bacilluria. Continuing bacilluria with streptomycin-sensitive bacteria has been reported (10), but in our experience these bacteria have been, without exception, highly resistant regardless of the use of concomitant alkali.

Bacterial resistance

The immediate factor in failure with streptomycin is development by bacteria of high degrees of resistance. This is observed frequently during treatment of all types of infection with streptomycin. The rapidity of development of resistance during the treatment of urinary tract infections is striking, and once the phenomenon is apparent, increasing dosage is of no avail. The *in vitro* sensitivity of bacteria before treatment is not a good guide to development of resistance. The number of organisms comprising the original population of the urine is probably the most important factor governing the appearance of this phenomenon (11). Bacteremia originating from bacteria that have become resistant in the urinary tract has been mentioned previously and emphasizes the importance of selection of the time for chemotherapy when various factors influencing cure are most favorable In accord with the permanence of streptomycin resistance produced by *in vitro* methods is the persistence of resistant bacteria in the urinary tract for long periods Many patients will continue to show resistant bacteria for at least 6 months after cessation of treatment. Some decrease in the degree of resistance, however, may occur over prolonged periods. Other patients may show the appearance of bacteria of the same or different species which are sensitive to streptomycin and probably represent new invaders

Anatomical abnormalities of the genito-urinary tract

The largest group of therapeutic failures occurs in patients in whom are present anatomical abnormalities of the genito-urinary tract that prevent free flow of urine and allow accumulation of residual urine. Such abnormalities are an indication for combined urological and medical approach. Foreign bodies in the urinary tract, such as calculi, indwelling catheters, suprapubic cystotomy or nephrostomy tubes, offer a convenient portal of entry for reinfection and predispose to development of bacteria resistant to streptomycin. Undrained abscesses represent foci that are inaccessible to chemotherapeutic agents and prevent clinical improvement or bacteriologic cure. Wounds with granulating surfaces or fistulas communicating

Cholera

Cholera is presumably caused by growth of *V. comma* and liberation of a toxin or toxins in the small intestine. Since vibrios do not invade the tissue, it is supposed that the toxin causes a great increase in the permeability of the enteric mucous membrane and perhaps the clinical "toxemia" to account for the signs and symptoms. As mentioned previously, the vibrios would seem, therefore, to be exposed ideally in the lumen of the bowel for attack by large amounts of streptomycin attained by oral administration.

Opportunity to test the therapeutic effects of streptomycin on cholera was afforded in the Chungking epidemic in 1945 (5), when ten patients were treated beginning on the first day of disease.

Three received 2.5 to 5 gm orally a day, in divided doses every 3 hours. Treatment was seldom continued beyond the fourth or fifth day, since recovery usually occurred before that time. All patients received routine injection of fluids for rehydration. Streptomycin was occasionally ejected in vomitus. Usually within 24 hours after beginning therapy, the stools, which had contained myriads of vibrios, were free from the organisms when examined microscopically, but in all instances *V. comma* could be cultivated from the feces during and for several days after therapy. No clinical benefit was noted as compared with patients treated by rehydration alone.

The effects of parenterally administered streptomycin in addition to that given orally were tested in seven patients. One received 3 gm intravenously and the others 4 gm intramuscularly daily in divided doses for 1 to 2 days. No benefit was observed.

The average duration of disease in patients who received streptomycin was 3 days as compared with 4.6 days among 56 who were not given the drug Since only 10 patients were treated, it is unsafe to ascribe the shortening of the disease to specific therapy.

Vibrios isolated from the stools of several patients before therapy varied greatly in their resistance to streptomycin. Some were sensitive to as little as 5 μg/ml of broth, others resisted more than 500 μg. Resistance to streptomycin bore no relation to the variety of the vibrios, whether they were of the AB (Ogawa), the AC (Inaba), or the ABC (Hikojima) types.

Typhoid

Typhoid, like cholera, theoretically should respond favorably to treatment with streptomycin. The extreme range of sensitivity of *S. typhosa* to streptomycin is reported as from 1 to 120 μg/ml of culture, but the usual range is from 1 to 16 (6) The latter amounts are easily attained

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CHAPTER 31

INTESTINAL INFECTIONS

Theoretically, streptomycin should be of value in the treatment of infections of the intestinal tract, since most of the bacilli involved are sensitive to its effects and amounts of streptomycin in excess of that needed for bacteriostasis in culture mediums usually can be attained in the blood and tissues after parenteral injection. Furthermore, certain enteric infections, like cholera and bacillary dysentery, both primarily local infections of the bowel, would seem to be ideal for treatment with streptomycin given orally, since by this route the drug is almost entirely excreted unchanged in the feces in accumulated amounts far in excess of those needed to kill the respective causative bacilli in culture medium. Oral administration of 4 gm a day provides 1 to 20 mg of streptomycin per gram of feces (1), the amount present varying with the dosage, the quantity of feces, and the rate of evacuation. When large amounts of streptomycin are present in the bowel, all sensitive bacteria greatly diminish in number or are eliminated; others are not (2) affected. Shortly after treatment is stopped and streptomycin disappears from the stool, the affected bacteria are rapidly reestablished.

Unfortunately, and for reasons unknown, streptomycin has failed generally to modify significantly the course of enteric diseases except bacillary dysentery, and not much space need be given this subject. To account for therapeutic failure in typhoid, it was presumed that typhoid bacilli often are intracellular and thus may be protected from the effects of the drug. This is not tenable, since *P. tularensis* is also found intracellularly, and tularemia is easily controlled with streptomycin. A more probable reason for therapeutic failure in both typhoid and cholera is the presence of strains of the respective bacilli, or variant forms of these strains, that either are initially resistant to bacteriostatic amounts of streptomycin or become so after exposure to it. Furthermore, it is known (3) that certain variant forms of *E. coli*, for example, require streptomycin for growth. In experimental studies (4) in mice inoculated with *S typhosa*, streptomycin in large dosage controlled infections in all animals, whereas smaller doses actually increased the fatality rate as compared with controls.

other patients (14) who received streptomycin orally and parenterally for 10 days, typhoid bacilli were not eliminated from the stools.

Infections Caused by Other Salmonella

Other bacilli of the *Salmonella* group are, in general, more resistant to streptomycin than is *S. typhosa*. The extreme range is reported (6) as inhibition by 4 to 120 μg; most strains are sensitive in the range of 4 to 32 μg/ml of broth.

Although favorable results from streptomycin therapy are occasionally reported, recovery, as in the case of typhoid, might have occurred without it. Generally, the results have been unsatisfactory. Nineteen patients with enteritis caused by *Salmonella* were reported on by the Committee on Chemotherapy (11). Of these, eight recovered, seven showed no effect, and five died. In three of five patients with bacteremia, the blood was cleared by streptomycin. In one patient with ulcerative colitis and superimposed *Salmonella* infection, temporary relief of symptoms followed each of three courses of therapy. Little noticeable effect was obtained from streptomycin in the treatment of five new born infants with enteritis caused by *S. typhimurium* (16). The bacilli disappeared from the stools during treatment but reappeared afterward. In one study (17), report is made of two patients with *Salmonella* dysentery who were said to have been cured. One, infected with *S. paratyphi*, failed to respond to sulfaguanidine but recovered after streptomycin was given orally in dosage of 0.5 gm. every 4 hours for a day and a half. The other patient, who was sick for 48 days with enteritis caused by *S. typhimurium* resistant to 50 μg of streptomycin, was given 0.1 gm. orally every hour and 0.25 gm. intramuscularly every 6 hours for 3 days. Pulaski also reported a patient infected with *S. paratyphi* (*S. paratyphosus* A) who was not aided by sulfaguanidine but recovered rapidly after receiving a total of 4 gm of streptomycin in doses of 0.5 gm. orally every 4 hours. Another severely sick patient with the same type of infection died in spite of treatment with 0.125 gm. of streptomycin intramuscularly every 3 hours.

References to several other case reports are given elsewhere (18).

Bacillary Dysentery (Shigellosis)

Although not many patients with shigellosis have been treated with streptomycin, the results have been better than for other enteric infections. Only two cases are mentioned in the report of the Committee on Chemotherapy (11). Both patients had received sulfonamides previously; both recovered after streptomycin treatment was begun in the third month in one and in the fifth month in the other patient. They received 1.5 gm of streptomycin intramuscularly daily for 5 days. Cultures of the stool, posi-

in the blood after parenteral injection and are far exceeded in the stool after oral therapy with the usual dosage.

The first opportunity for a clinical trial of streptomycin on typhoid came during an epidemic in 1945 (2). Five patients were treated: one orally, two intramuscularly, one intravenously, and one both orally and intravenously. The oral or intramuscular dosage varied from 1 to 4 gm daily. Three patients recovered during treatment, but recovery could be ascribed to spontaneous improvement; the others were not favorably influenced. In subsequent studies (1) five more patients were treated with similar equivocal results. In one patient who recovered, the causative typhoid bacilli resisted amounts of streptomycin in broth larger than the amount attained in the blood; in another in whom infection was not controlled, 22 μg of streptomycin was present per milliliter of blood yet the bacillus was sensitive *in vitro* to only 9 μg. Bacteremia persisted during therapy. In several instances when tests were made, there was no evidence that the causative bacilli had become more resistant to streptomycin as a result of exposure to it. Parenteral therapy had no influence on the presence or number of typhoid bacilli in the feces, but during oral therapy typhoid bacilli and other bacteria were diminished greatly in number or were eliminated as long as therapy was continued and streptomycin was present in bacteriostatic amounts in the feces. *S. typhosa* and other suppressed bacteria reappeared in the stools soon after streptomycin was discontinued.

Several other reports of treatment with streptomycin of one or more patients (7–14) describe similar results, with the general conclusion that streptomycin is of no value in altering the clinical course or the fatality rate in typhoid. It is not known why *S. typhosa* persists in the blood when amounts of streptomycin are present in excess of that needed to kill the bacilli in culture medium, but the intracellular location of the organism alone cannot be held responsible. It is more likely that streptomycin-resistant variant forms are present, persist, or even multiply, or that the environment in the body protects otherwise sensitive bacilli from the antibiotic

As might have been predicted from early studies (2) attempts to rid carriers of typhoid bacilli have been unsuccessful. Typhoid bacilli though eliminated from the stool while streptomycin was present there in large amounts after oral administration, reappeared when treatment was stopped

In one study (15) of carriers, no changes in the number of typhoid bacilli in the stools were noted during intramuscular administration of streptomycin, as would be expected. During oral therapy of .03 gm every 3 hours for eight doses, the stools of two of three carriers were freed from *S. typhosa*, but the bacilli reappeared 12 hours after treatment was stopped. In the

other patients (14) who received streptomycin orally and parenterally for 10 days, typhoid bacilli were not eliminated from the stools.

Infections Caused by Other Salmonella

Other bacilli of the *Salmonella* group are, in general, more resistant to streptomycin than is *S. typhosa*. The extreme range is reported (6) as inhibition by 4 to 120 μg; most strains are sensitive in the range of 4 to 32 μg/ml of broth.

Although favorable results from streptomycin therapy are occasionally reported, recovery, as in the case of typhoid, might have occurred without it. Generally, the results have been unsatisfactory. Nineteen patients with enteritis caused by *Salmonella* were reported on by the Committee on Chemotherapy (11). Of these, eight recovered, seven showed no effect, and five died. In three of five patients with bacteremia, the blood was cleared by streptomycin. In one patient with ulcerative colitis and superimposed *Salmonella* infection, temporary relief of symptoms followed each of three courses of therapy. Little noticeable effect was obtained from streptomycin in the treatment of five new born infants with enteritis caused by *S. typhimurium* (16). The bacilli disappeared from the stools during treatment but reappeared afterward. In one study (17), report is made of two patients with *Salmonella* dysentery who were said to have been cured. One, infected with *S. paratyphi*, failed to respond to sulfaguanidine but recovered after streptomycin was given orally in dosage of 0.5 gm. every 4 hours for a day and a half. The other patient, who was sick for 48 days with enteritis caused by *S. typhimurium* resistant to 50 μg of streptomycin, was given 0.1 gm. orally every hour and 0.25 gm. intramuscularly every 6 hours for 3 days. Pulaski also reported a patient infected with *S. paratyphi* (*S. paratyphosus* A) who was not aided by sulfaguanidine but recovered rapidly after receiving a total of 4 gm of streptomycin in doses of 0.5 gm. orally every 4 hours. Another severely sick patient with the same type of infection died in spite of treatment with 0.125 gm. of streptomycin intramuscularly every 3 hours.

References to several other case reports are given elsewhere (18).

Bacillary Dysentery (Shigellosis)

Although not many patients with shigellosis have been treated with streptomycin, the results have been better than for other enteric infections Only two cases are mentioned in the report of the Committee on Chemotherapy (11). Both patients had received sulfonamides previously; both recovered after streptomycin treatment was begun in the third month in one and in the fifth month in the other patient. They received 1.5 gm of streptomycin intramuscularly daily for 5 days. Cultures of the stool, posi-

tive before treatment, became negative afterward. A patient of Morgan and Hunt (12) with dysentery caused by *Sh. sonnei* was given 1 gm. of streptomycin orally five times a day beginning the fifth day of disease. Recovery occurred within 12 hours and *Shigella* disappeared from the stools Pulaski and Amspacher (17) reported uniformly good results in six patients of various ages with dysentery caused by the sonnei, flexner W, flexner V and W, and newcastle types of *Shigella*. One case had lasted 8 days; the rest, 7 to 15 months as chronic dysentery. Sulfonamide therapy had failed previously in each case. Streptomycin was given orally in doses of 0.05 gm every hour to 1 gm every 6 hours, and intramuscularly from 0.1 gm every 2 hours to 0.4 gm every 4 hours for 4 to 16 days depending on the size of the patient and other circumstances. Two were treated orally alone. Good results were obtained whether streptomycin was given orally, parenterally, or by both routes. Dysentery bacilli disappeared from the stool in most instances. No recurrences were observed.

The largest series of persons treated with streptomycin together with untreated ones for control was reported by Hardy and Halbert (19). This report includes carriers as well as patients with dysentery.

Streptomycin in sweetened milk was given orally four times daily over a 3-day period to thirty-seven persons 5 to 15 years old Twenty persons received 3 gm of streptomycin and seventeen received 6 gm, during the 3-day period. Patients whose *Shigella* resisted sulfonamide therapy were included. During and shortly after treatment, shigellas rapidly decreased in number in the stools from all patients and were gone by the sixth day. Other streptomycin-sensitive bacteria also decreased in number. At the third week, shigellas reappeared in eight persons' stools.

Many previous studies have shown sulfadiazine to be effective in treating bacillary dysentery and to rid carriers of *Shigella*. Sulfadiazine is at present the drug of choice so far as cost is concerned, but since streptomycin is equally effective it may be preferable. No toxic effects were noted in any patients who received streptomycin by mouth. Streptomycin is indicated in patients known to be sensitive to sulfonamide compounds or in severely dehydrated patients, and if the bacilli are, or become, resistant to sulfadiazine. The dosage recommended at present is 1 to 4 gm daily, in divided doses orally, for 3 to 16 days depending on circumstances. Since dysentery bacilli may lodge deep in the tissues, some observers have recommended combined oral-intramuscular therapy, to be included in the same range of dosage.

Many more studies under controlled conditions are needed before final evaluation of streptomycin for treatment of bacillary dysentery can be made.

Infectious Diarrhea of Infants

The cause of this often fatal disease of infants is unknown. Numerous bacteria of the *Shigella*, *Escherichia*, *Salmonella*, and *Proteus* groups and others have been suspected. The concensus at present favors an unknown virus or viruses as the cause. Nevertheless, Pulaski and Amspacher (17) treated with streptomycin twelve infants with infectious diarrhea and believed that good results were obtained in several. Two died, and another suffered a recurrence of the disease. The dosage, given orally, was 0.3 to 0.6 gm daily per kilogram of body weight. These workers recommended further trial in the treatment of the disease with the following regime: isotonic solution of sodium chloride, blood or plasma given intravenously for dehydration and the shock-like state, early oral feeding 75 to 125 calories per kilogram of body weight if there is no vomiting, and streptomycin in minimal dosage of 0 5 gm per kilogram of body weight. In smaller infants and in critical cases, minimal doses up to 1 gm per kilogram of body weight per day were advised. Treatment should be continued for 1 week.

Idiopathic Chronic Ulcerative Colitis

Since the cause of this disease is unknown, antibacterial therapy at present is empirical except as a measure to control secondary infection, to alter the flora of the intestine, or to prepare the bowel for surgical operation. Sulfonamide compounds, both soluble and relatively insoluble, and penicillin are of little value (20), but streptomycin theoretically should be helpful because of its properties of attacking pathogenic gram-negative bacilli and certain other bacteria that inhabit the intestine, and because large amounts of it accumulate in the bowel during oral therapy.

Reports of the use of streptomycin in only about 40 cases of chronic ulcerative colitis (21) make it difficult to assess the value of the drug. Observers seldom have treated more than a few patients, and some of the published reports give no detailed information. Controlled studies with untreated patients in a disease like ulcerative colitis are hard to arrange satisfactorily because of the great variation in severity and duration of the disease and its natural tendency to remission and relapse. Thus far, as would be expected, opinion as to the value of streptomycin therapy varies. Some regard its use unjustified (20), some as of doubtful value (10), and others recommend it to reduce fever and toxicity during exacerbations (17, 21, 22) or to prepare the bowel for surgical operation The results in general are not encouraging, and a number are similar to those reported in one of the earliest observations, namely, "during therapy the number of stools lessened, but the fever persisted unchanged." Other workers had similar

experience (23). In certain instances, however, temporary remission undoubtedly has occurred in severe attacks while the drug was given either parenterally, orally, or both. The few good results thus far reported have apparently resulted from treatment in the early period of the first attack. It is impossible, however, in such instances to know whether the disease would have become chronic if streptomycin had not been given.

Of two patients with first attacks of colitis on whom good results were reported, one treated in the sixth week (24) with intramuscular injections of 0.5 gm every 4 hours began to recover within a day or two; the other (25) treated during the third month also recovered promptly after receiving 0.1 gm intramuscularly every 3 hours. Of nine cases reported by Pulaski and Amspacher (17), significant improvement occurred in only three during active episodes. Most of the patients were given streptomycin orally in doses of 0.25 to 1 gm every 3 to 6 hours and intramuscularly in doses of 0.2 to 0.5 gm every 3 to 4 hours. Most of the patients with chronic colitis were said to have received some, if only temporary, benefit from therapy. In another series of seven cases (11) no beneficial effect was recorded.

No reports of the use of streptomycin for regional ileitis have been encountered.

Streptomycin in Preparation for Intestinal Surgery

The striking diminution in the number of bacteria in feces during oral administration of streptomycin for typhoid as noted by Reimann, Elias, and Price (2) suggested use of the drug to reduce the bacterial content of the bowel before surgical operation (1). Even though the flora is suppressed only as long as streptomycin is present in sufficient amount in the bowel, such a temporary reduction is desirable during intestinal surgery.

In a small series of patients (1), 1 to 6 gm of streptomycin daily in divided doses every 3 hours by mouth gave 600 to 16,000 μg/gm of feces. Colon bacilli were eliminated in most instances. In one patient, however, despite daily 5-gm doses of streptomycin and the presence of 2,000 to 16,000 μg/gm of feces, colon bacilli and other bacteria persisted. These colon bacilli resisted more than 200 μg of streptomycin per milliliter of broth.

During oral therapy, bacteria sensitive to streptomycin, including *S. fecalis*, usually begin to diminish 12 hours after the first dose and when at least 600 μg of streptomycin per gram of feces is present. Growth is usually reestablished 24 to 48 hours after the last dose, or when the stool no longer contains bacteriostatic amounts of the drug.

Streptomycin given orally is poorly absorbed, and no toxic effects have been reported. In this respect it is superior to the sulfonamide compounds. In one study it was superior to succinylsulfathiazole in reducing the number

of *S. fecalis* and *E. coli* in feces (26). Some observers recommend a combination of a sulfonamide compound and streptomycin (27).

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Fundamentally the treatment of recurrent cholangiohepatitis should be the operative removal of the predisposing factors. Thus the common duct should be reconstructed when a stricture exists; common duct stones should be removed, and internal fistulae should be excised. In general, operative procedures should not be attempted during an acute exacerbation of cholangiohepatitis unless continuing fever makes this necessary Clinically, it has long been recognized that biliary tract surgery can be performed with greater safety when the bilirubinemia becomes stabilized than when the bilirubinemia is either increasing or decreasing in intensity. This is generally true regardless of whether the plateau of bilirubinemia is at a high or a low level. The clinical or subclinical jaundice associated with cholangiohepatitis is in part due to ductal obstruction and is in part an expression of parenchymal cell injury of the liver. There is little doubt that cholangiohepatitis produces more liver cell damage than simple obstruction alone.

Streptomycin is used in the preoperative preparation of patients with cholangiohepatitis and in the treatment of cholangiohepatitis when operation for one reason or another is not possible. It is used alone or in combination with penicillin. A total daily dosage of 1.0 to 2.0 gm in divided doses is administered intramuscularly every 6 hours for 4 to 6 days before operation. When penicillin is also used, a total daily dose ranging from 400,000 to 1;000,000 units a day is administered. The clinical course of a patient with cholangiohepatitis treated with streptomycin and penicillin is shown in figure 72. This patient had had acute exacerbations of cholangiohepatitis at intervals for 2 months. Penicillin, 400,000 units total daily dose, apparently had no effect upon the disease; however, following the addition of streptomycin therapy his temperature remained normal.

Following intramuscular injection the concentration of streptomycin found in the bile is approximately half the concentration of the blood serum (4). Figure 73 shows the concentrations of streptomycin found in the blood serum and bile of a patient who had received 0.6 gm of streptomycin in 200 ml of physiological saline solution by the intravenous route. Following the administration of 0.125 gm of streptomycin intramuscularly every 3 hours for 24 hours, the concentration of streptomycin in the common duct bile was 10 units. According to Zaslow, Counseller, and Heilman (5), streptomycin, like penicillin, is less readily excreted by the liver in the presence of liver damage. Since recurrent cholangiohepatitis over a period of months or years may produce considerable hepatic damage, the effectiveness of antibiotic therapy may be considerably reduced. In the presence of complete biliary obstruction, penicillin and streptomycin are not found in the bile. Although the nature of cholangiohepatitis makes

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CHAPTER 32
CHOLANGIOHEPATITIS AND PERITONITIS

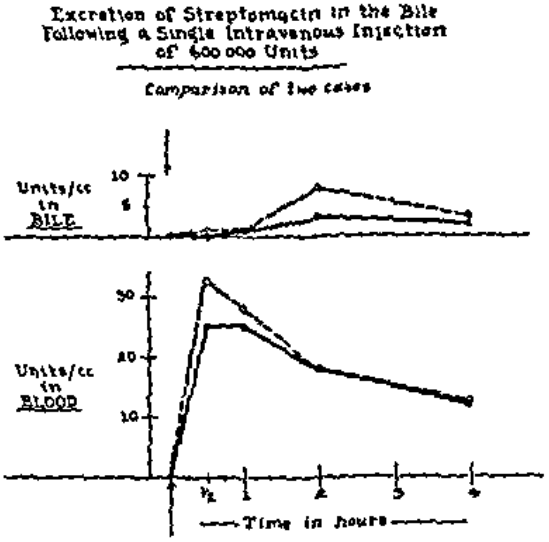
CHOLANGIOHEPATITIS

Streptomycin is used in the treatment of cholangiohepatitis as an adjunct to other forms of therapy. Although the nature of the clinical manifestations make it difficult at times to evaluate the effect of any therapeutic agent on the clinical course of the disease, apparent beneficial effects following the use of streptomycin in the treatment of cholangiohepatitis have been reported (1, 2, 3). The acute phase is usually heralded by a chill, which is followed by an elevation of temperature, often as high as 102° to 105°F. The elevation of temperature seldom persists for more than a few hours unless suppuration occurs. The temperature may then remain normal for irregular periods—sometimes days and not infrequently months—without specfic therapy. Associated with the chill and fever, there is an elevation of the serum bilirubin content which may or may not be sufficient to produce clinical jaundice. In the presence of frequent exacerbations of infection and considerable parenchymal injury, persistent clinical jaundice is usually present.

The most frequent predisposing factor of cholangiohepatitis is some degree of obstruction of the common bile duct either by stricture or by calculi. Occasionally cholangiohepatitis is produced by the regurgitation of intestinal contents into the biliary ductal system. The most common causes of regurgitation of intestinal content into the biliary tract are internal fistulae between the biliary and gastro-intestinal tracts and loss of function of the sphincter of Oddi. Either of these conditions will allow intestinal contents with the contained bacteria to gain access to the biliary tract. When calculi are present in the common bile duct or in its precursors, infection coexists Apparently the presence of calculi in the common bile ducts favors the growth of bacterial organisms. Culture of the common duct bile in the presence of common duct calculi will frequently show the presence of bacteria In our experience *E. coli* and streptococci were each present in 50 per cent of the patients who had bacteria in the common duct bile. Staphylococci, *Ps aeruginosa, Pr. vulgaris*, clostridia, diphtheroids, and Salmonella were found in occasional cultures.

imals. In studies of experimental intestinal strangulation in dogs, Blain and Kennedy (9) found that the dogs treated with massive doses of penicillin survived 50 to 100 hours as compared to an average survival time of 36 hours for the untreated animals. Davis *et. al.* (10) found that 100 per cent of rabbits treated with very large doses of streptomycin survived experimental strangulation of the bowel, whereas all of untreated animals died.

On the basis of the experimental studies cited, it would appear that streptomycin does have some beneficial effect in the treatment of experimental peritonitis, even though the effect in some experiments was not very striking. In each of these animal experiments, either a greater number of the treated animals survived or the average survival time of the treated groups was greater than in the untreated groups.



The largest reported clinical experience with the use of streptomycin in the treatment of peritonitis is that of Pulaski, Seeley, and Matthews (11) These investigators have analyzed the results of antibiotic therapy in sixty-three patients to whom streptomycin was administered for the treatment of peritonitis. Twenty-one of these patients received streptomycin alone, and forty-two received streptomycin and penicillin. Recovery was thought to be more smooth and more rapid when the combination of antibiotics was used Resolution of clinical evidences of peritoneal suppuration in the streptomycin-treated patients was of approximately the same pattern as was seen in the patients receiving in excess of 800,000 units of penicillin a day Consistently beneficial effects were not observed with streptomycin

it difficult to determine quantitatively the effectiveness of streptomycin in the treatment of the disease, we have repeatedly seen evidence of its usefulness. In the absence of complete biliary obstruction, streptomycin inhibits the growth of bacterial organisms in the bile.

Peritonitis

Because most types of clinical peritonitis show a mixed type of bacterial infection, considerable interest was aroused in the possible effectiveness of streptomycin in the treatment of clinical peritonitis.

In 1,000 infections treated with streptomycin and reported by the Committee on Chemotherapeutics and Other Agents of the National Research Council (6) there were fifty-three patients with peritonitis. In this report

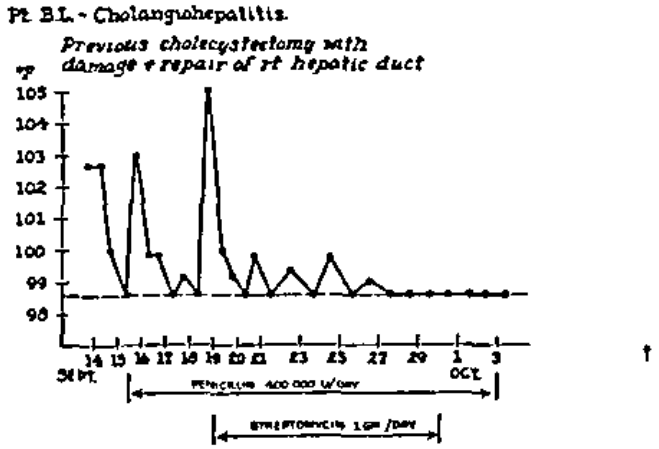


Fig. 72. Penicillin therapy over a period of 3 5 days failed to prevent an acute exacerbation of hepatocholangitis. When streptomycin was given in addition to penicillin, acute exacerbations did not occur, and the temperature became normal after 4 days of combined therapy

attention was directed to the difficulty in evaluating the effectiveness of a therapeutic agent in a disease which had so many variabilities concerned with recovery from the infection The greatest single cause of peritonitis in this group of patients was appendicitis, there were twenty-three patients with appendiceal peritonitis. Of these twenty-three patients treated with streptomycin, nineteen survived

Murphy, Ravdin, and Zintel (7) found that systemically administered streptomycin increased the survival rate of dogs in which peritonitis had been produced experimentally. Silvani, Rothenberg, Warmer, Amluxen, and McCorkle (8) did not feel that streptomycin was effective in the treatment of experimental peritonitis even though their two treated groups of animals lived an average of 92 and 75 hours as compared to the average length of survival of only 39 hours for the untreated control groups of an-

imals. In studies of experimental intestinal strangulation in dogs, Blain and Kennedy (9) found that the dogs treated with massive doses of penicillin survived 50 to 100 hours as compared to an average survival time of 36 hours for the untreated animals. Davis *et. al.* (10) found that 100 per cent of rabbits treated with very large doses of streptomycin survived experimental strangulation of the bowel, whereas all of untreated animals died.

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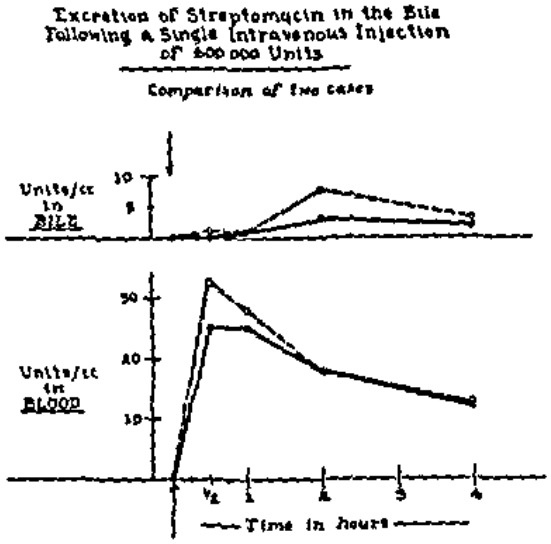

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alone when this antibiotic was administered in total daily doses of 2.0 gm or less. In the experience of these investigators, the patients who received a total daily dose of 3 0 gm of streptomycin or a combination of 2.5 gm of streptomycin and 480,000 units of penicillin daily had the most satisfactory postoperative convalescence. They concluded that although streptomycin is not a panacea, yet it has a valuable place in the treatment of peritonitis.

Zintel and Zinsser (12) compared the relative effectiveness of streptomycin, penicillin, and a combination of penicillin and streptomycin in the treatment of experimental peritonitis. In a control group of thirty dogs in which peritonitis was produced, only one dog survived the 10-day period of observation. Of fifteen dogs treated with streptomycin alone, four, or 27 per cent, of the animals survived the 10-day period of observation. With combined penicillin and streptomycin therapy, twelve of twenty dogs, or 60 per cent, survived, and with penicillin therapy alone, ten of fifteen dogs, or 66 per cent, survived a similar period of observation. The percentage of survivals with penicillin therapy alone was not significantly different from the percentage of survivals with combined penicillin and streptomycin therapy. Penicillin alone resulted in a survival rate which was twice that observed with streptomycin alone.

On the basis of clinical and experimental observations it would appear that massive doses of penicillin are effective in the treatment of peritonitis. Although streptomycin is effective in the treatment of peritonitis, apparently it is less effective or no more effective than massive doses of penicillin Addition of streptomycin to massive doses of penicillin in the therapy of peritonitis has not, in clinical or experimental studies, shown a greater degree of protection than would be expected if massive doses of penicillin alone were administered

Although the usefulness of streptomycin in conjunction with penicillin therapy has not been demonstrated either clinically or experimentally in the treatment of peritonitis, it has been our practice to use both antibiotics, in the treatment of clinical peritonitis. We have continued to use the combination of antibiotics for two reasons: first, it is always possible that the bacterial organisms in the peritoneal cavity of an individual patient might not be sensitive to penicillin even in massive doses; second, on the basis of the observations of Carpenter et al. (13) it would appear that increased bacterial resistance to antibacterial agents is less likely to occur if several antibacterial agents are used simultaneously. In addition to operation when indicated and to supportive methods of therapy, it has been our practice to treat patients with peritonitis with approximately 1,000,000 units of penicillin and either 1.0 or 2 0 gm of streptomycin daily Patients so treated have usually responded satisfactorily.

Oral Administration of Streptomycin

Streptomycin administered orally is not absorbed in any appreciable amount from the gastro-intestinal tract, and it is effective in reducing the number of viable bacteria in the feces. Oral streptomycin is used prophylactically in patients who are to have elective surgery of the large bowel (14). In a limited number of patients whom we have studied, streptomycin was more effective in reducing the total number of viable organisms of the coliform, *S. faecalis*, and clostridial groups than was sulfasuxidine or sulfathaladine. The patients studied were without evidences of intestinal obstruction. They received a low-residue diet and no enemas or laxatives during the period of observation. In most of these patients the maximum antibacterial effect was achieved in about 8 days of therapy, regardless of whether streptomycin or the sulfonamides were used. The incidence of initially resistant organisms or organisms that became resistant during the period of observation was approximately the same for the three antibacterial agents used. Rowe *et al.* (15) have also noted the beneficial effect of oral streptomycin, but in their experience a number of the organisms in the feces rapidly developed resistance to the effect of streptomycin. They suggested that patients be prepared for operation by administering sulfasuxidine 5 days before operation and then by adding streptomycin therapy 3 days before the intended operation.

Streptomycin can be taken orally in any liquid food. A daily dose of 1.0 gm is apparently sufficient. A dose of one-quarter gm is administered four times a day. It should be remembered that sulfasuxidine, sulfathalidine, or streptomycin may reduce the amount of vitamin K available from bacterial synthesis to any given patient. There is no evidence that streptomycin or the sulfonamides cause an inability of absorption of vitamin K on the part of patients receiving this vitamin either in their food or in a purified form. If an adequate amount of vitamin K is available to the patient either in the diet or in a supplementary form, there should be no evidence of a vitamin K deficiency resulting from sulfonamide or streptomycin therapy.

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CHAPTER 33

PREVENTION OF WOUND INFECTION

A solution of streptomycin of the proper concentration can be injected into tissues surrounding wounds or can be swabbed on wounds to reduce bacterial contamination without interfering with healing, because the bactericidal concentration of the drug is less than that which kills freshly injured tissues. Further, because streptomycin destroys bacteria not killed by penicillin or the sulfonamides, a combination with either one cross-fires at the mixed bacterial flora of wounds and is effective against all but a few resistant strains Hence, streptomycin mixtures should be used to prevent development of infection in contaminated wounds. These facts were first demonstrated in the experimental animal and later evaluated clinically by statistical methods. Veterinarians have already used streptomycin by local injection for treatment of wounds in animals.

The demonstration that a mixture of streptomycin with other antibacterials prevents infections in wounds suggests that at last an effective "subcutaneous" antiseptic is at hand and that a new era of surgery has been entered.

Since Lister's time, a substance that would kill bacteria and not interfere with healing has been sought. Until the introduction of the sulfonamides, antiseptics killed both bacteria and the tissues, and wounds failed to heal. Hence, antiseptics were never used in the treatment of wounds, except to clean the surrounding skin. Instead, infection was prevented in operative wounds by freeing the hands, instruments, and linens of bacteria and, in the case of traumatic wounds, by cutting away the injured tissues with their content of bacteria and foreign bodies The wound was then irrigated with saline. Strangely, antiseptics have always been used, however, for the treatment of cuts and bruises, although the physiological principles governing the treatment of cuts and bruises do not differ from those governing the treatment of larger wounds. But the hysteria to do something drastic and the insistence of First Aid Manuals impel the accidentally wounded, or those in attendance, to pour highly colored antiseptics into traumatic wounds instead of washing them with running water and thus removing surface bacteria.

Shortly after the sulfonamides were introduced and the demonstration was made that they would not interfere with the growth of cells in tissue culture, it was optimistically believed that here finally was the ideal subcutaneous antiseptic. There were many reasons why these high hopes were not justified. Sulfanilamide, sulfathiazole, and sulfadiazine were only bacteriostatic and therefore could not kill bacteria promptly; blood interfered with their action; and they affected only gram-positive cocci. Further, it was forgotten that only in certain concentrations were these substances nonlethal to cells. Thus when powders of these sulfonamides were put in wounds to dissolve, the more soluble sulfanilamide and sulfathiazole created too great a concentration locally, and the large undissolved portion of the less soluble sulfadiazine acted as a foreign body residue.

Later, penicillin also failed as the ideal subcutaneous antiseptic, not because of cell toxicity or lack of bactericidal power in the presence of blood, but because it destroyed only gram-positive cocci and was, in turn, rendered useless by persisting gram-negative bacilli. How this came to be understood is of interest. After the demonstration by Florey and Cairns (1) that topical application of penicillin would not hasten the resolution of established infection in wounds, the reason for this failure was sought. Penicillinase, a potent substance that destroyed penicillin, was found to be elaborated by and was finally isolated from the gram-negative bacilli (2) *E. coli*, *Ps. aeruginosa* and *Pr. vulgaris*, which invariably came to inhabit the pus of infected wounds as contaminants of fecal origin, and with enormous capacities to spread, elaborated the penicillin inhibitor and, in consequence, they were not destroyed by penicillin (3). For this reason, the best way to rid the wound of penicillinase seemed to be to destroy the gram-negative bacilli by using with penicillin another antibacterial that would be more or less specific for these organisms

Until this conclusion was reached, however, the need for an antibacterial that would destroy only gram-negative bacilli had received little attention In the "antiseptic era," antiseptics theoretically destroyed the gram-negative bacilli as well as all others. But urologists who paid more attention to the significance of this group of bacteria had found these organisms difficult to destroy by any means Later, when the sulfonamides were widely used, there seemed little reason to seek an adjunct antibacterial substance to kill the gram-negative bacilli, because failures were attributed to para-aminobenzoic acid (4) rather than to bacterial infection.

Moreover, whether the gram-negative bacilli were harmful or were simply secondary growers in pus had often been discussed. It was pointed out that gram-negative bacilli dominated the flora of pus only in the later stages, and despite their presence, granulations grew abundantly and con-

traction of the wound took place (5). Besides, in areas like the perineum where these bacilli are always abundant, excellent healing of wounds occurred, and this excellent healing was pointed to as further evidence that the bacilli did no harm. Some even believed, and still believe, that an enzyme secreted by the gram-negative bacilli helps to liquefy slough, that the discharge of pus ceases as soon as slough is liquefied, and that excellent healing occurs thereafter, although gram-negative bacilli are still present. Schmidt *et al.* (6), however, have demonstrated that only one or two of the least common variety of gram-negative bacilli secrete a collagenase, and therefore, if hastening of liquefaction is a reality, abundance of pus must be responsible for the enzymes.

The opponents of the thesis of the benignity of gram-negative bacilli point out, on the other hand, that the profuse discharge produced in response to these organisms depletes the patient's serum protein, prevents the spread of growing skin grafts, and even liquefies successfully transplanted grafts (7). They emphasize also that gram-negative infections do not clear up in tissues like bone, which do not liquefy readily. Lastly, it must be admitted that the exudative phase of healing of the perineal wound might be shorter if the gram-negative bacilli were not present

Thus the finding that the gram-negative bacilli inactivate penicillin crystallized the need for an antibacterial capable of destroying them without destroying tissues not because they themselves were harmful but because the gram-negative bacilli influenced infection and the healing of tissues by encouraging the return of secondary invaders.

This need was not easily filled, however. Before the isolation of streptomycin by Waksman (8), no satisfactory substance was available to kill the gram-negative bacilli. Acetic acid, thought to be specific, only decolorized them All the common antiseptics except parachlorophenol failed, and this substance was found to be toxic to freshly injured tissues, though not to granulations. An antibiotic exhibiting low cell toxicity was difficult to find because the metabolism of the gram-negative bacilli closely resembles that of the cells (9) Streptothricin, first isolated by Waksman (10), was definitely toxic.

Streptomycin in a concentration of 200 units/ml is nontoxic to fresh growing cells and is bactericidal. This concentration was, of course, selected by experimentation, by chosing a unitage that would kill bacteria promptly and yet not kill tissues. Although only the bactericidal activity of this concentration is shown in comparison to other antibacterials, in the discussion the tissue toxicity tests used to select the proper concentration are described more completely because this phase of the problem is too often neglected.

Laboratory Tests

In vitro tests of bactericidal activity

For effective local chemotherapy, antibacterial substances should possess prompt bactericidal activity in the presence of blood and pus.

To find how short a contact was necessary to kill various bacteria, the filter paper, blood agar-plate method of testing antiseptics was adopted. A 24-hour culture of the particular bacteria to be investigated was streaked on a blood-agar plate, which was incubated for 3 hours. Then a piece of filter paper, dipped in the solution of antibacterial substance, was placed on the agar for 10 minutes. This contact time was selected as representing the extreme limit of "prompt action." The control area was treated with filter paper soaked in saline or was merely overlaid with filter paper. The plate was then incubated for 24 hours.

Bacteria grew abundantly over the control area and over the rest of the plate where there was no antibacterial substance; but where the treated filter paper was in contact with the plate and immediately surrounding this area, no growth appeared, the agar remaining clear. This clear zone varied considerably in extent. Apparently, the variations could be attributed to the sensitivity of the bacteria, because the duration of contact and the concentration of the antibacterial substance were constant in each test (fig. 74).

The bactericidal activity of 200 units of streptomycin per milliliter as determined by this method is shown in table 66. It should be noted that not only gram-negative bacilli but some gram-positive bacteria were killed as well.

For comparison, the effect of 200 units of calcium penicillin is shown in table 67. Here the bactericidal activity is limited sharply to the gram-positive group

In table 68 the bactericidal activity of a combination of streptomycin, 200 units per milliliter, and 5 per cent sulfamylon is shown. This combination has a wide bacterial spectrum and therefore encounters few resistant organisms.

These tests show that streptomycin promptly kills bacteria in the presence of blood and that, in addition to killing gram-negative bacilli, it destroys a different group of gram-positive bacteria from those affected by penicillin Further, when streptomycin is combined with an antibacterial like sulfamylon, the mixture has a very wide range of bactericidal activity and encounters few resistant organisms. The sulfamylon combination is stable and therefore preferable to the similar mixture with penicillin.

TABLE 66

Bactericidal activity of streptomycin, 200 Mg/ml, 10-minute contact

DATE OF TEST	STRAIN OF BACTERIA	INHIBITION OF GROWTH OF BACTERIA IN AREA CONTACTED BY PAPER	INHIBITION ZONE ABOUT THIS AREA
May 17, 1945	*E. coli*	++++	++
June 12, 1945	*E. coli*	++++	++
June 22, 1945	*E. coli*	++++	+
June 13, 1945	*Pr. vulgaris*	++++	+
June 22, 1945	*Pr vulgaris*	++++	+
May 17, 1945	*Pr. vulgaris*	++++	++++
June 12, 1945	Staphylococcus (coagulase positive)	++++	++
June 22, 1945	Staphylococcus (coagulase positive)	++++	++++
June 12, 1945	*B. pyocyaneus*	+++*	0
June 22, 1945	*B. pyocyaneus*	+++*	0
May 17, 1945	*B. pyocyaneus*	++†	0
Oct 10, 1945	*S. hemolyticus*	0	0
Oct. 10, 1945	Green streptococcus	0	0

* Spread in from edges

† Overgrown in 36 hours.

TABLE 67

Bactericidal activity of calcium penicillin, 200 Mg/ml, 10-minute contact

DATE OF TEST	STRAIN OF BACTERIA	INHIBITION OF GROWTH OF BACTERIA IN AREA CONTACTED BY PAPER	INHIBITION ZONE ABOUT THIS AREA
May 21, 1945	Staphylococcus (penicillin-resistant strain)	+	0
May 24, 1945	Staphylococcus	++++	+++++
June 1, 1945	Staphylococcus	++++	++++
June 1, 1945	Staphylococcus (penicillin-resistant strain)	0	0
Aug. 14, 1945	*E coli*	0	0
Aug. 14, 1945	*Pr vulgaris*	±	0
Aug. 14, 1945	*Ps. aeruginosa*	0	0
Aug. 14, 1945	*Ps aeruginosa*	0	0

Tissue toxicity tests

Streptomycin in various concentrations was tested for toxicity against cells growing in tissue culture, on rabbits for the amount of irritation caused to the conjunctiva of the eye, for its capacity to damage subcuta-

neous blood vessels, for its capacity to destroy muscle on injection, to cause peritoneal adhesions, and to influence the rate of healing of a wound on the ear.

TISSUE CULTURE TESTS

In these tests, the concentration of streptomycin was kept constant throughout and the substance was continuously in contact with cells, a severe test of toxicity. In the method employed, previously described by Simms (11), adult cells that possess different growth requirements from those of embryonal cells were used.

Epithelial cells and fibroblasts from adult animals were incubated in serum ultrafiltrate to keep them alive. Then they were transposed to

TABLE 68

Bactericidal activity of a combination of streptomycin, 200 µg/ml, sulfamylon, 5 per cent, and sodium benzoate, 0.5 per cent

DATE OF TEST	STRAIN OF BACTERIA	INHIBITION OF GROWTH OF BACTERIA IN AREA CONTACTED BY PAPER	INHIBITION ZONE ABOUT THIS AREA
May 25, 1945	Staphylococcus	++++	++
June 12, 1945	Staphylococcus	++++	++++
June 12, 1945	Staphylococcus	++++	++++
June 1, 1945	Staphylococcus	++++	++
June 1, 1945	Staphylococcus	++++	++
May 17, 1945	Staphylococcus and *E coli*	++++	++
June 12, 1945	*E coli*	++++	++
June 24, 1945	*E coli*	++++	++
June 13, 1945	*Pr vulgaris*	++++	++
June 22, 1945	*Pr vulgaris*	++++	+
June 12, 1945	*Ps aeruginosa*	++++	+
June 22, 1945	*Ps aeruginosa*	++++	+
Oct 10, 1945	*S hemolyticus*	++++	+++
Oct 10, 1945	Green streptococcus	++++	+

chicken plasma to grow, and streptomycin in various concentrations was added When the concentration was toxic, the cells failed to grow or grew poorly. A concentration of 200 units/ml was found to be mildly inhibitory; 100 units/ml was inert The combination with sulfamylon was toxic.

Eye tests. These tests indicated the concentration of agent that would irritate the delicate tissues of the conmjunctiva Vasodilatation, edema, and exudate were produced Contact with the tissues was short, and some dilution occurred.

Two or three drops of solution were placed in one eye of the rabbit, and a similar amount of saline was put in the opposite eye. The immediate reaction of blood vessels and their condition 30 minutes later were noted. The state of the blood vessels and the amount of edema were observed 24 hours afterward.

Sometimes even saline caused immediate injection of the blood vessels. Except for those treated with calcium penicillin, 200 units/ml, and the

FIG. 74. Bacteriocidal activity of (a) sodium sulfadiazine; (b) streptomycin, (c) sulfamylon plus streptomycin.

mixture of streptomycin, 200 units, and sulfamylon, 5 per cent, all treated conjunctivae had returned to their normal appearance at the end of ½ hour. Streptomycin alone, 200 units/ml, did not affect the cornea. After 24 hours, all tests were negative

Muscle injections. The injections were made to indicate the capacity of agents to cause inflammatory reaction and fibrosis in muscle In these tests, the substance was in contact with the tissues between the time of injection and the dilution and absorption of the agent.

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Eye tests. These tests indicated the concentration of agent that would irritate the delicate tissues of the conmjunctiva. Vasodilatation, edema, and exudate were produced. Contact with the tissues was short, and some dilution occurred.

was free of exudation and had bright red granulations at the proper level to allow the next epithelium to extend across. Delayed healing prolonged the index beyond 7.5 days, and correspondingly, the appearance of the granulations deviated from average. The best index obtained was 5.6 days.

A concentration of 400 units of streptomycin per cubic centimeter of base definitely slowed the rate of healing—index 8.5. On the other hand, 200 units gave an index of 6.4, and 200 units with 5 per cent sulfamylon gave an index of 5.7. With 1,000 units of penicillin and 200 units of streptomycin per milliliter, the index was 7.3. In other words, 200 units of streptomycin alone or in combination with penicillin or 5 per cent sulfamylon was demonstrated to be innocuous to healing tissues. Since these concentrations were bactericidal, it at once suggested that the combination with sulfamylon should be an ideal subcutaneous antiseptic.

Prevention of infection in experimental wounds

To prove that the streptomycin-sulfamylon combination would prevent infection in wounds, a method previously devised of producing 100 per cent infected wounds in experimental animals was employed. Failure to cure these infections by treatment with other agents has been reported elsewhere (12). The wounds were made as follows: Under anesthesia, a defect 3 cm. square was cut in the skin of the back of the rabbit just lateral to the spinal column. The loose subcutaneous tissues and fascia were excised, and the underlying muscle was vigorously crushed with a Kocher clamp. The wound was then contaminated wth bacteria in floor dirt or with a 24-hour broth culture of a specific bacterium.

One group of wounds was treated with streptomycin and sulfamylon immediately after contamination; a second group, 3 hours after contamination; and a third group, after an interval of 12 hours.

The treated wounds were washed with the combination of streptomycin and sulfamylon without debriding the crushed tissue and then 15 to 20 ml was injected in the tissues about the wound. The wound was covered with vaselined gauze, and a protective dressing and jacket was applied to the animal. The control wounds received no treatment except vaselined gauze.

All wounds were inspected 4 days after infliction to determine whether infection had occurred. The amount of exudate and the appearance of the tissues were noted. Color photographs were taken of the wound to record the character of the healing. To determine the quantity of bacterial contamination, a cotton swab was saturated with secretions from the wound and smeared on a blood-agar plate, which was incubated for 24 hours. A similar swab was also smeared directly on a glass slide and given

Five to 10 cc of the various antibacterial substances at different concentrations were injected in the muscles of one hind leg of the rabbit. To eliminate error in locating the site later, the needle was inserted anteriorly to the bone at a definite distance above the knee joint. In the opposite leg, the same amount of saline was injected. The animals were killed 24 and 48 hours later, and the tissues were examined grossly and microscopically.

Calcium penicillin, 200 units/ml, produced some slight edema and exudation for the first 24 hours; thereafter, no reaction was observed. Streptomycin, 200 units, and sulfamylon, 5 per cent, individually produced a reaction comparable to that of calcium penicillin. The mixture of streptomycin, 200 units, and sulfamylon, 5 per cent, produced no more edema or leucocytosis than did the single substances. After 24 hours no unusual tissue reactions were observed.

INSTILLATION IN PERITONEAL CAVITY

Twenty cubic centimeters of calcium penicillin, 200 units; sulfamylon, 5 per cent; streptomycin, 200 units; and a combination of the last two were injected into the peritoneal cavity of the rabbit to determine whether they would cause adhesions. The cavities were examined 10 days later and were found to be free of adhesions.

INFLUENCE ON RATE OF HEALING

To estimate the influence of the concentration of streptomycin on the character and the length of the exudative phase of healing of an open wound and on the rate of regeneration of new tissues thereafter, the wound was treated by two methods: first, the antibiotic was applied in a base, second, it was applied in solution for a time and then the wound was dressed with a base to prevent drying. This base*, cholesterized petrolatum, had been previously determined to be innocuous both to freshly injured and to granulation tissues

The wound was made on the ear of a rabbit, leaving a small island of skin at the center The wound was photographed every day, and the photograph was projected and a tracing made. From these tracings, growth of the island was recorded Exudation, the character of the granulations, and the time when the island began to grow were noted.

To express the rate of healing, an index was calculated in terms of the number of days required for the exudative period and to produce a definite amount of new tissue The average index was 7.5 days, meaning that 3.5 days was required for the exudative period and an additional 4 days was required to regenerate 2 mm of new epithelium A wound with this index

*(Amerchol)

TABLE 69

Effect of streptomycin-sulfamylon mixture on experimental wounds

RABBIT NUMBER	CONTAMINANT	TREATMENT	GROSS APPEARANCE AFTER 4 DAYS	BLOOD PLATE CULTURE ON 4TH DAY
		Untreated controls		
640	Floor dirt	None	Grossly infected	Good growth of bacteria
642	Staphylococcus culture	None	Wound very dry and necrotic	Thick culture of *Staph.*
644	Staphylococcus culture	None. Vaselined gauze	Wound full of pus	Thick culture
648	Floor dirt	None Vaselined gauze	Wound full of exudate	Good growth of bacteria
660	Floor dirt	None Vaselined gauze	Wound full of dead tissue and pus	Good growth of bacteria
		Treated series		
641	Floor dirt	Immediately treated	Wound clean	No growth
643	Staphylococcus culture	Immediately treated	Tissues, in general, healthy Good healing obtained later	Scattered colonies
649	Floor dirt	Immediately treated	Clear bloody exudate. Edematous connective tissue.	Two or three colonies
650	Floor dirt	Immediately treated	Wound clean and moist	No growth
659	Floor dirt	Immediately treated	Healthy and red, with some dead tissue	One colony
		Delayed treatment		
645	Staphylococcus culture	3 hrs after trauma treated with mixture	Considerable pus	Good growth of bacteria
646	Staphylococcus culture	3 hrs. after trauma treated with mixture	A little pus and dead white tissue	Thickly scattered colonies, 200+
647	Floor dirt	3 hrs after trauma treated with mixture	Dead tissue and pus under skin edges	Good growth of bacteria
656	Floor dirt	24 hrs. after trauma, debrided and treated with mixture	No gross pus	No growth
651	Floor dirt	24 hrs. after trauma, debrided and treated with mixture	No gross pus	One colony

a Gram stain. The character of the exudate and the number of bacteria present were noted.

Subsequent to the first dressing, both the wounds that had become infected and those that had not were treated every other day by swabbing the surface with the combination of streptomycin and sulfamylon to determine whether a rapid resolution of the established localized infection could be brought about or whether the protection against infection continued.

Five wounds treated with the mixture of streptomycin and sulfamylon immediately after contamination remained free of infection. Five wounds not treated, filled with pus (table 69). Grossly, the tissues of the nontreated wounds were gray, necrotic, and swollen (fig. 75). The tissues of the treated wounds showed no evidence of infection; they were bright red, and the crushed tissue had disappeared (fig. 76). A small amount of clear fluid was present. Direct smears from the infected wounds showed that every leucocyte contained bacteria, and there were many extracellular microorganisms as well. Smears from the treated wounds showed numerous leucocytes, but only an occasional one contained bacteria, and none were extracellular. Blood plates taken from the untreated wounds showed heavy concentrations of bacteria (fig. 77). Those taken from the treated wounds on the contrary, showed only occasional colonies of bacteria or no growth (figs. 78 and 79).

Moreover, when infection was prevented, the wounds remained uninfected, regardless of subsequent treatment. The granulations continued to be bright red. Contracture of the surrounding tissues and regeneration of new tissues took place rapidly. Figure 80 shows such a wound almost healed on the 15th day. Also, additional treatment of the infected wounds did not cause rapid resolution, even though the bacteria remained susceptible to the antibacterial agent. Figure 81 shows that in the infected wounds contraction took place although pus and slough were still present.

In contrast to the success obtained with immediate treatment, infection occurred in the group of five wounds treated 3 hours after contamination. Four of these were grossly purulent; the other did not have frank pus but contained considerable fluid. The blood plate from this as well as those from the other four showed characteristic dense growths of bacteria. Typical results are shown in table 69.

Again, the bacteria recovered from these wounds remained susceptible to the action of the combination and hence, their acquired resistance would not account for the failure to prevent infection. Blood smears taken from the wounds just before they were treated, that is, 3 hours after contamination, showed that actually fewer bacteria were present on their surfaces at the time of treatment than when they were first contaminated. Varia-

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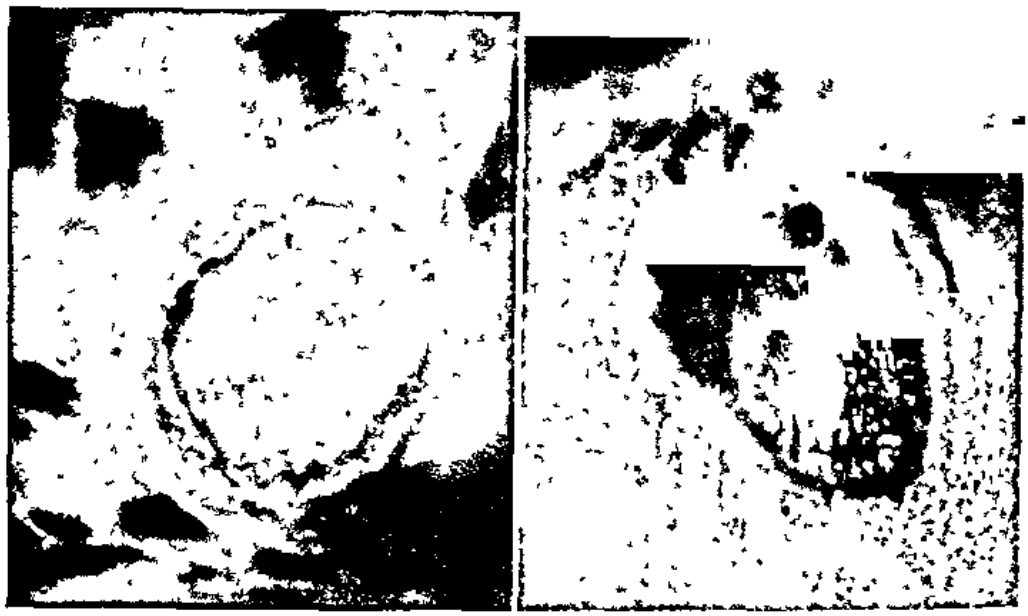

FIG 75 Untreated experimental wound, after 4 days
FIG 76 Treated experimental wound, after 4 days.

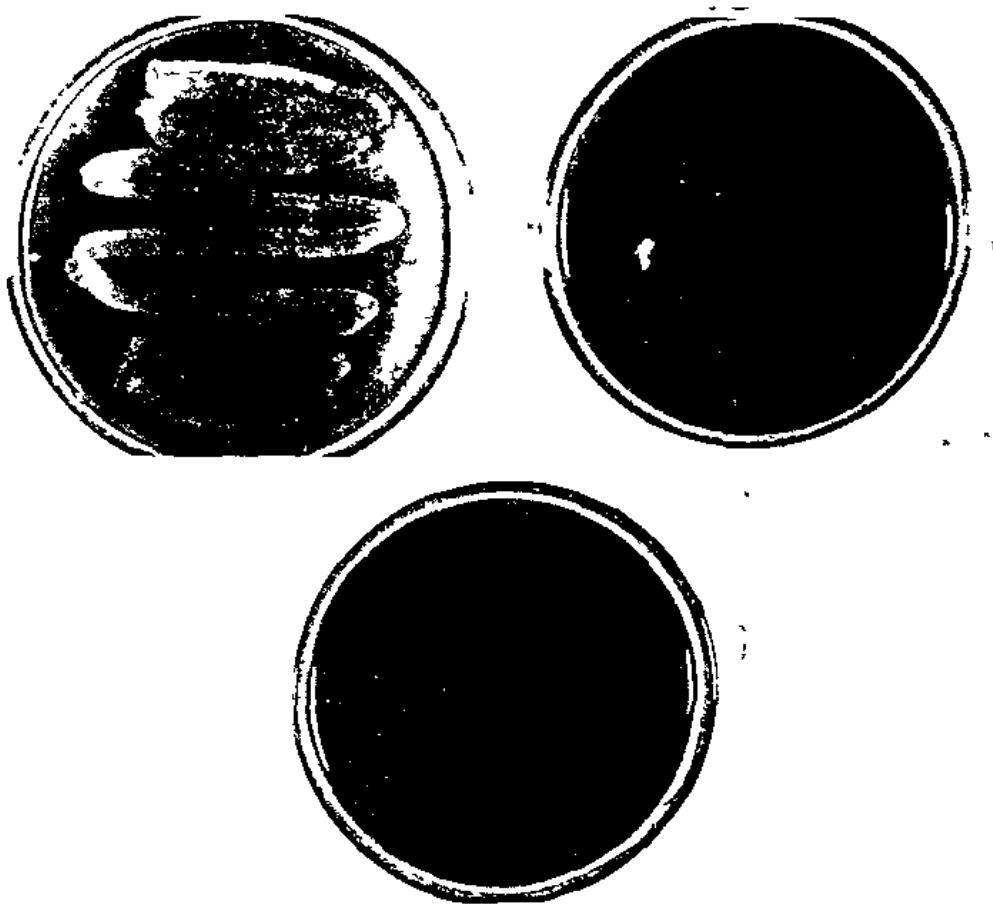

FIG 77 Blood plate taken from untreated experimental wounds
FIG 78 Blood plate taken from treated experimental wound showing only occasional colonies
FIG 79 Blood plate taken from treated experimental wound showing no growth.

tion in penetration would not seem to offer a logical explanation for the failure because the mixture of antibacterials was injected in both instances. In an attempt to find an explanation, biopsies were taken of the tissues about the wound. It was observed that many leucocytes had invaded the tissues at the end of 3 hours and already contained many bacteria.

The combination also failed to prevent infection from developing when given 12 hours after contamination. The bacteria remained susceptible to the action of the combination of streptomycin and sulfamylon, although they had definitely increased in numbers. Again, biopsy of the tissues showed many leucocytes which contained bacteria.

Lastly, one of the 3-hour wounds and one of the 12-hour wounds were

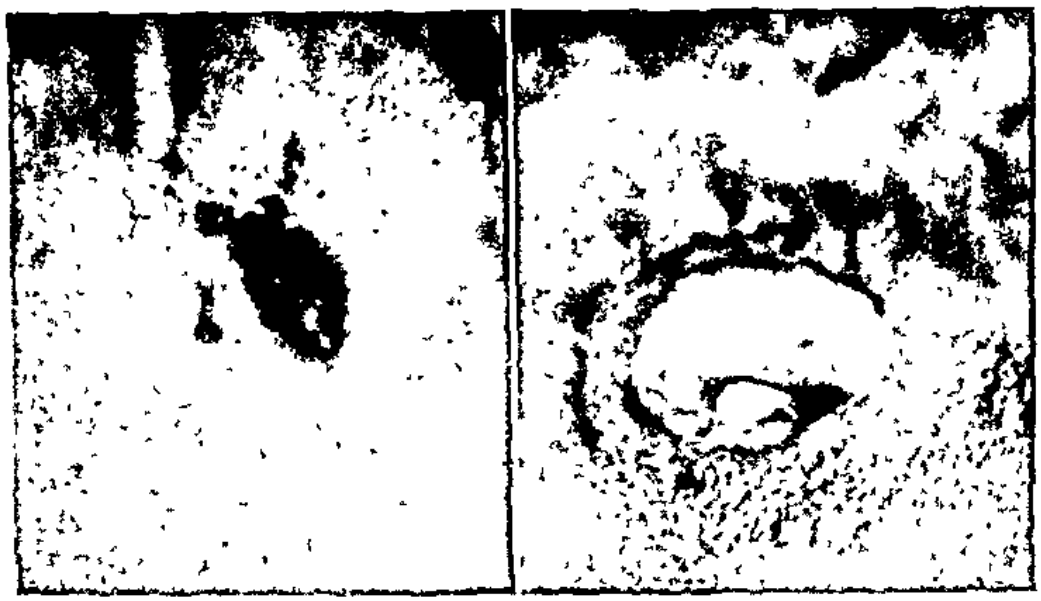

FIG. 80 Experimental wound almost healed on the 15th day
FIG 81 Infected experimental wound showing contraction in the presence of pus and slough

treated by debridement and then irrigated and injected with the combination of streptomycin and sulfamylon. Infection was prevented in both wounds. A 12-hour wound debrided but not treated became infected.

With no pronounced increase in the number of bacteria in the wound, with the bacteria remaining susceptible to the action of streptomycin-sulfamylon, and with the amount of traumatized tissue unchanged, some other reason must be offered to explain why infection was not prevented when the wound was treated 3 or more hours after injury. The possibility that the microorganisms were deeper in the tissues seems untenable because the solution was injected. For the same reason, deposition of fibrin, preventing diffusion of the solution, does not seem to offer a plausible explanation. Similarly, dilution by edema fluid below the bactericidal concentration does not provide a logical answer because an excessive amount of solution was used.

The best explanation seems to be that the bacteria were no longer extracellular and, therefore, the solution failed to make contact with them. Phagocytosis placed these bacteria within the leucocytes. Both Wright (13) and Flemming (14) showed that ingestion of bacteria by leucocytes did not necessarily destroy them. In fact, they made cultures from washed leucocytes and grew the contained bacteria. Further, Flemming believes that leucocytes wandering to the surface of wounds die, and undoubtedly they are killed in many ways. Thus if the leucocyte disintegrates before the bacteria are destroyed, then the microrganisms may be free to contaminate the wound again. If, therefore, phagocytized bacteria are to be destroyed, either the antibacterial substance must penetrate the leucocyte or it must be present in a bactericidal concentration when the bacteria escape from the leucocyte. Both conditions are difficult to accomplish.

The 3-hour period was selected at random. Consequently, no statement can be made as to whether the combination of streptomycin and sulfamylon would fail if used within this interval. Similarly, nothing can be said about the effects of delayed use beyond the 12-hour period, for then new difficulties are added. Phagocytosis continues, the number of bacteria increases, and the bacteria begin to invade tissues and cause necrosis. Necrotic tissue not only shields the bacteria from the action of the antibacterial substance but also provides ready nutrition for the injured microorganisms Kirby, Dull, Fulton, and Zintel (15) have recently confirmed that the use of streptomycin in experimental wounds prevents infection.

Prevention of Infection in Clinical Wounds

With this thorough laboratory background on the efficacy and the limitations of streptomycin in the prevention of wound infection, clinical trials were begun To delimit the problem, only clean-contaminated operative wounds were treated The clean contaminated wound is made clean but becomes contaminated with bacteria during the operation either from the opening viscera or from purulent collections. In other words, the clean-contaminated wound provides nearly ideal conditions to prove that the wound can be decontaminated by chemotherapeutic means. The mixture of streptomycin and sulfamylon was used because of its better range of bactericidal activity Blood plates made before and after treatment of the wound showed that many colonies of *E. coli* were still present, but they were always very much smaller in size if a 10 minute contact was obtained. With the Staphylococcus the colonies were always reduced in number, and were also reduced in size The traditional method of swabbing the wound without injecting the solution into the surrounding tissues, was adhered to. As much as 200 ml has been put into the peritoneal cavity at one time without ill effect

In 477 wounds treated in this manner, not a single untoward effect has been encountered. No general drug reaction has been observed, nor have any complications of healing been noted that could be attributed to use of the solution. In other words use of the solution in the wound appears to be harmless.

The percentage of infection in this series was 1.3 per cent (table 70). In contrast, Meleney (16) found that in the years 1938–1941 the percentage of infections developing in clean-contaminated wounds without general sulfonamide or antibiotic therapy varied from 8–9.2 per cent at Presbyterian Hospital in New York City (table 71). During this time the paren-

TABLE 70

Prevention of wound infection in clean-contaminated wounds treated with streptomycin-sulfamylon mixture

NUMBER OF WOUNDS		NUMBER INFECTED	PERCENTAGE OF INFECTION
477	Total	6	1 3
126	Large bowel resections	3*	
39	Gastrectomies	0	
11	Peritonitis general	1	
90	Appendectomies frankly purulent localized	1†	
15	Pilonidal sinuses	1	
4	Tuberculous nodes	0	
192	Miscellaneous		

* Large bowel failure—pus came from drainage tract

† Appendectomy failure—wound in abdominal wall became infected

teral use of first the sulfonamides and then penicillin was introduced but if the sevenfold reduction in incidence of infection in this type of wound was caused by the combined parenteral use of penicillin and local treatment with streptomycin and sulfamylon, then this is an important gain. Meleney's analysis of a large number of infections in traumatic wounds, showed that neither parenteral administration of the sulfonamides or penicillin reduced the incidence (16) Unfortunately, no statistics are available for the incidence of infection in clean-contaminated wounds treated by penicillin alone Hence, it is impossible to tell whether parenteral therapy with penicillin alone, local use of the solution of streptomycin and sulfamylon, or the combination of the two can be given credit The significant fact is the marked reduction in the incidence of infection since parenteral antibiotics and the combination of streptomycin and sulfamylon have been used locally.

One hundred and eighty-five traumatic wounds have been treated with the combination of streptomycin and sulfamylon solution, used in the

wound and to clean the surrounding skin, as the only means of decontamination Whenever there was grease on the skin, a grease solvent was used before the streptomycin and sulfamylon solution was supplied. Only one trivial infection occurred. The solution of streptomycin and sulfamylon has also been used to saturate gauze used to pack open wounds that were to be treated later by delayed primary closure. The wound was again washed with this solution just before the primary closure. Thirty-two treatments of this type have been carried out without an infection. It is hoped that this form of therapy will reduce to almost zero the incidence of infection with delayed primary closure.

Clinical experience with local treatment of the established infection with the combination of streptomycin and sulfamylon has paralleled that found experimentally; namely, resolution of infection was not hastened. That the established infection will not be helped has been emphasized since the beginning of this work (17), yet experienced workers like Florey, Ross,

TABLE 71

Healing of clean-contaminated wounds without sulfonamide or antibiotic therapy (16)

	CASES	TOTAL INFECTION
		per cent
1938	774	8.0
1939	866	8 7
1940	964	8 3
1941	859	9 2

and Turton (18) have written that the combination of streptomycin and sulfamylon has been recommended for the mixed infection. They have also tried it clinically for this purpose and, of course, found it useless. Repeated admonitions on this point, given to our own staff, go unheeded and their results are generally unsuccessful also. An adjunct chemotherapeutic agent that will liquefy necrotic tissue and aid in the destruction of the bacteria in leucocytes must be used in the established infection

On the other hand, whenever granulations free of slough become overgrown or remain undergrown with a mixed flora of bacteria they can be quickly returned to their optimal condition by treatment with dressings saturated with the solution of streptomycin and sulfamylon

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Chapter 34

CLINICAL WOUND INFECTIONS

Literature Review

Experimental

In an experimental controlled study in which both gram-negative and gram-positive infections were introduced into wounds in dogs, Kirby, Dull, Fulton, Price, and Zintel (1) found that streptomycin administered intramuscularly produced a negative blood culture and reduced toxicity and mortality, and that streptomycin in an ointment base (one application of 50,000 units), either immediately upon infection or 8 hours later, appeared to produce the same results. The results of a study in rabbits, made by Howes (2), before that reported by Kirby, led to the conclusion that streptomycin combined with sulfamylon was effective in preventing infection when injected immediately into the wound; but, contrary to Kirby's finding of effectiveness when treatment was delayed, Howes found that an injection 3 or more hours after infection was ineffective.

Clinical

The literature contains few reports of clinical studies. White (3) and Hirshfield, Buggs, Pilling, Bronstein, and O'Donnell (4), working separately made substantially similar findings, namely, that in granulating wounds there was clinical improvement, but that, probably because of development, during treatment, of streptomycin-resistant organisms, this antibiotic, *per se*, topically applied or intramuscularly administered, did not consistently eliminate the infecting organisms. Wilson (5) reported favorable results with streptomycin (saline solution alone or combined with intramuscular therapy) in a wide variety of infections in fifty-five patients, as a cover for skin-grafting, and especially in superficial lesions if necrotic tissue is removed. Streptomycin-resistance accounted for some case failures Keefer, Blake, Lockwood, Long, Marshall, and Wood (6), reporting on

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surgical wound infections in six patients, found improvement on use of parenteral or topical streptomycin therapy. Florey, Ross, and Turton (7) reported sterilization of five out of six predominantly gram-negative chronic sinus tracts; the exception was attributed to the presence of *S. aureus* and *S. hemolyticus*, subsequently eliminated with topical penicillin applications. Brooke (8) reporting on the effects of topical application of 0.25 per cent parachlorophenol and 0.5 per cent streptomycin in a carbowax base in treating ten cases of ulcers, burns, and infected wounds, stated that the results were generally good.

United States Army Experience

In the following analysis of 200 cases of streptomycin-treated nontuberculous infections of soft tissues in hospitals of the United States Army,

TABLE 72

*Bacteriology of 172 cases of wound suppuration**

ORGANISMS	TOTAL	PURE	MIXED
S aureus	131	34 (26%)	97 (74%)
Beta hemolytic streptococci	21	2 (10%)	19 (90%)
Nonhemolytic streptococci	34	3 (9%)	31 (91%)
E coli	39	12 (31%)	27 (69%)
Kl pneumoniae (Friedlander's)	30	8 (27%)	22 (73%)
Pr vulgaris	41	3 (7%)	38 (93%)
Ps aeruginosa	17	2 (12%)	15 (88%)
Others	3	2 (66%)	1 (34%)

* 28 closed lesions not cultured

findings corroborated and supplemented those in a small number of previously reported cases (9).

The bacterial flora cultured from 172 cases is listed in table 72. Twenty-eight acute infected closed wounds, presumably coccal in origin, were not cultured Two-thirds of the lesions had a mixed flora with *S. aureus* predominating. One hundred thirty-five cases received streptomycin alone. The remaining sixty-five were being treated also with penicillin, even though penicillin alone had been ineffective. Fifty-two per cent of all cases benefited. In 11 per cent the response was doubtful; in the remaining 37 per cent the response was negligible. Response to therapy was indicated by the pathogenesis of the lesion. The pathogenesis fell into three groups: (a) cellulitis, (b) superficial and deep abscesses, and (c) miscellaneous. Descriptions of the response by the various groups follow:

Group I—Cellulitis (Table 73)

These lesions occur over a large area and may suppurate. Of the fifty-three cases treated, 88 per cent benefited with either streptomycin alone or with streptomycin plus penicillin. The 12 per cent listed as doubtful or failures were acute flares in scar tissue. Adjuvant measures were limited to needle-aspiration or stab-wound drainage. The results thus compared favorably with those obtained with penicillin alone. The usual dose of streptomycin was 0.5 gm four times a day for 4 to 7 days. Untoward reactions were rare with this dosage.

Group II—Superficial and Deep Abscesses (Tables 74 and 75)

These lesions are grouped together because their response to therapy is similar.

The abscesses in the fifty-one cases (table 74) occurred in traumatic

TABLE 73

Group I—Results of therapy in 53 wounds with cellulitis

DIAGNOSIS	STREPTOMYCIN ALONE				STREPTOMYCIN WITH PENICILLIN			
	Number of cases	Benefited	Doubtful	Not benefited	Number of cases	Benefited	Doubtful	Not benefited
Cellulitis	18	17	0	1	8	8	0	0
Cellulitis with suppuration	21	17	1	3	6	6	0	0
Total	39	34	1	4	14	14	0	0

wounds, the urinary system, and as furuncles and carbuncles. Best results were obtained in infected traumatic wounds, where 61 per cent were benefited Improvement in the urinary tract lesions was only 42 per cent, and with furuncles and carbuncles only 19 per cent.

For adequate response, free drainage and appropriate excision of dead tissue were necessary Failure with streptomycin was associated with recurrence of infection in scar, dead tissue, constant source of reinfection from fistula or sinus, and inadequate drainage.

The eighty-one postoperative wound infections (table 75) accounted for 40 per cent of the cases Of these lesions, 80 per cent developed under the prophylactic use of penicillin Of forty-six cases treated by streptomycin and penicillin, 42 per cent were benefited, 12 per cent were doubtfully benefited, and 46 per cent were unaffected. The results with either penicillin plus streptomycin or streptoycin alone were similar.

The postoperative infections included twenty-two of the abdominal wall, twelve of the flank, six of the perineum, and forty-one wound revi-

sions. Half of the nonhealing abdominal wounds were complicated by fecal fistulae. The usual daily dose of streptomycin was 2.5 gm for 7 to 10 days.

TABLE 74

Group II—Results of therapy in 51 surgical abscesses

DIAGNOSIS	NUMBER OF CASES	BENEFITED	DOUBTFUL	NOT BENEFITED
Traumatic wounds	23	14 (61%)	3 (13%)	6 (26%)
Carbuncles and furuncles	16	3 (19%)	4 (25%)	9 (56%)
Urinary system	12	5 (42%)	1 (8%)	6 (50%)
Total	51	22 (43%)	8 (16%)	21 (41%)

TABLE 75

Group II—Results in 81 postoperative wound infections

DIAGNOSIS	NUMBER OF CASES	BENEFITED	DOUBTFUL	NOT BENEFITED
Abdominal wall	22	10 (45%)	2 (10%)	10 (45%)
Flank	12	5 (42%)	1 (8%)	6 (50%)
Perineum	6	1 (16%)	1 (16%)	4 (68%)
Wound revisions	41	18 (43%)	6 (14%)	17 (43%)
Total	81	34 (42%)	10 (12%)	37 (46%)

TABLE 76

Group III—Results of therapy in 15 miscellaneous wound infections treated with streptomycin

DIAGNOSIS	NUMBER OF CASES	BENEFITED	DOUBTFUL	NOT BENEFITED
Ecthyma	2	0 *	2	0
Decubitus ulcer*	2	0	0	2
Chronic burns (2°–3°)	3	0	0	3
Buerger's disease	3	0	0	3
Sinus and fistula	4	0	0	4
Acute tenosynovitis	1	0	0	1
Total	15	0	4	11

* One case treated with streptomycin and penicillin

Poor results accompanied fecal fistulae; foreign bodies, as bone chips, plastics, silk, and cotton or metal; urinary fistulae, and sloughing perineal wounds

Group III—Miscellaneous Wound Infections (Table 76)

The miscellaneous infections gave the least response to streptomycin. Thus unsatisfactory results occurred in cutaneous gangrene, Buerger's disease, chronic tenosynovitis, chronic burns, chronic ulcers, and chronic fistulae. In the four cases doubtfully benefited, two cases of ecthyma and two of decubitus ulcer, streptomycin apparently initiated improvement, but bacteria persisted and wounds failed to heal. The wounds were closed by excision and grafting.

Topical application of streptomycin as ointments, packs, and irrigations has been ineffective in our hands. Bacteria, especially cocci, pyocyaneus, and proteus, in infected sloughing and granulation tissue, may become streptomycin-fast within 1 or 2 days. If bacteria are not eliminated from wounds after two or three applications of streptomycin, further local treatment is useless.

Summary

1. Over-all benefit in 200 streptomycin-treated nontuberculous infections of soft tissues was 52 per cent.

2. Patients who were treated also with penicillin showed no greater benefit.

3. Topical application of streptomycin was of no value.

4. Ninety per cent of the patients with cellulitis benefited from streptomycin therapy.

5. Superficial and deep abscesses required adjuvant measures for beneficial response Of the patients with infected traumatic wounds, 61 per cent were benefited. Furuncles and carbuncles improved in only 19 per cent of the cases, and infections connected with the urinary tract were suppressed in only 42 per cent of the cases.

6. In postoperative wound infections, 42 per cent were benefited. Factors which contributed to poor results are listed.

7. Miscellaneous infections (cutaneous gangrene, Buerger's disease, chronic tenosynovitis, chronic burns, chronic ulcers, and chronic fistulae) showed least response to streptomycin.

8. The recommended dosage is 2 0 to 2.5 gm streptomycin *q i.d.* for 4 to 10 days. Toxic reactions are uncommon with this dosage.

CONCLUSIONS

Streptomycin is of benefit in surgical infections, but it is not a panacea. It is most effective in cellulitis; but only moderately effective in cases of adequately drained superficial and deep abscess. The most beneficial effects of therapy are observed in acute infections when the drug is given early in the disease. Thus, the principle uses of the drug are with gram-

positive and mixed infections in which there has been no response to maximal doses of penicillin within 48 to 72 hours. Earlier use of streptomycin is indicated if *in vitro* evidence of penicillin resistance can be obtained. In chronic suppurating lesions the drug is useful only as an adjuvant to drainage and section of compromised tissue for the suppression of residual bacteria. In our experience, topical application of streptomycin has not given good results.

Two-thirds of open pyogenic surgical infections are polybacterial. Careful bacteriologic analysis of the wound bacteria and culture-sensitivity testing are necessary prerequisites to successful streptomycin therapy. This antibiotic has inhibitory action on most aerobic gram-negative and gram-positive surgical organisms, but wide variation in susceptibility is observed and initially streptomycin-fast organisms are found (fig. 82)

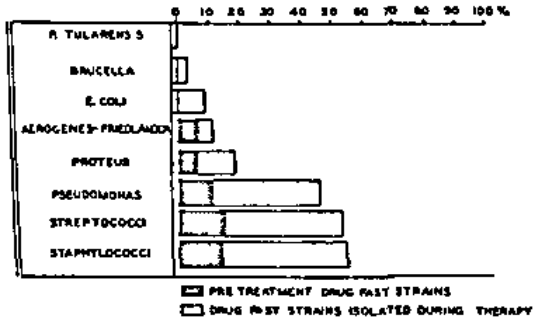

FIG. 82. Streptomycin fastness *in vivo*

Drug-fastness is a common factor in unsatisfactory therapeutic responses. For this reason, the drug should be used only when properly indicated. The requisites for successful streptomycin therapy are:

1. Streptomycin-sensitive organisms.
2. A dosage of at least 2.0 gm a day.
3. Adequate blood supply.
4. Absence of necrotic tissue.

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"degeneration bodies" which have lost their normal staining reaction disappear rapidly, giving the impression of a rapid diminution of total number of bodies present.

Streptomycin resistance

Of the patients in this series, 4 to 5 per cent proved resistant to streptomycin. One such patient received daily doses of 1 gm for 28 days, 1 5 gm for 41 days, 4 gm for 9 days, and another course of 4 gm daily for 10 days. In view of the fact that some patients responded to as small a total dose as 3 3 and 5 gm, it is amazing that the one patient failed on a total of 165.5 gm.

FIGS. 84 and 85. Granuloma inguinale before and after treatment with streptomycin.

Another patient who proved resistant had received a total dose of 100 gm, 2 gm daily for 30 days and 4 gm daily for 10 days. Some tissue from this patient was used for experimental reproduction of the disease in a volunteer. A small, circumscribed experimental lesion developed in this volunteer. It failed to respond to two courses of streptomycin, 4 gm a day for 5 days. The streptomycin-resistant cases, incidentally, have responded to oral aureomycin therapy (11).

CONCLUSIONS

Streptomycin has proved a most useful drug in the therapy of granuloma inguinale. Many patients with this disease have responded to as little as

treated with 4 gm for 5 days frequently showed complete healing within 1 to 2 weeks after cessation of therapy. The patients with very extensive lesions responded similarly, except for residual areas, which progressed to eventual healing within a few weeks without additional therapy. Extragenital lesions (four cases) healed more rapidly than did those in the genital area.

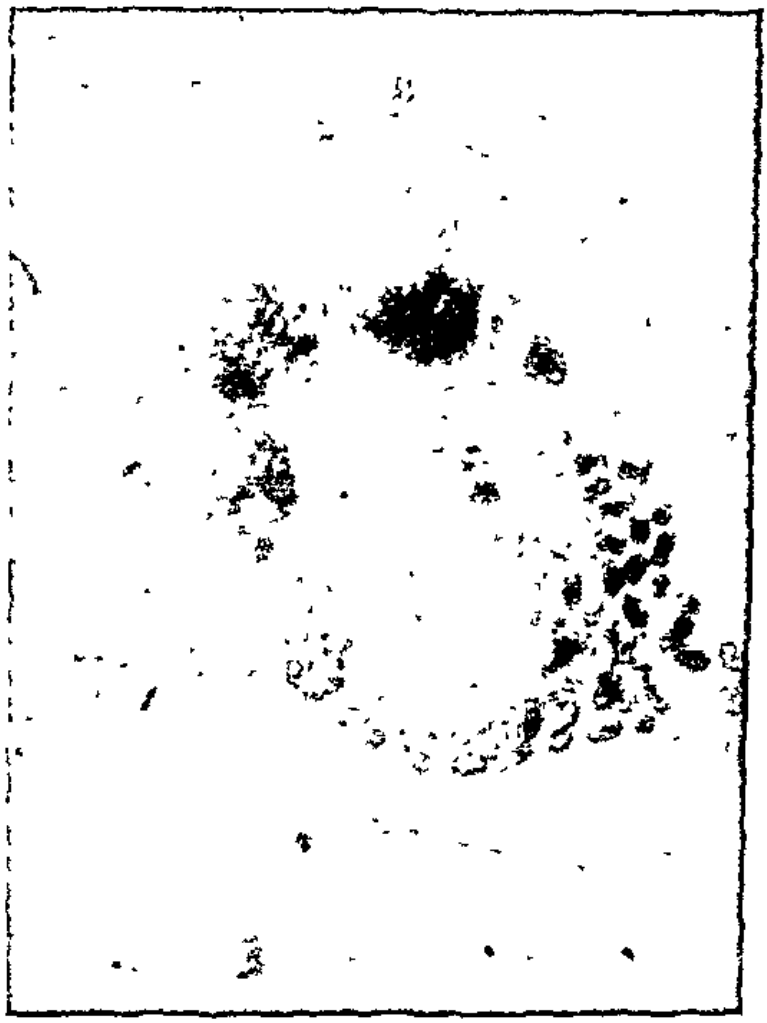

FIG. 83. Donovan bodies

Fate of the Donovan bodies

Daily smears from the lesions showed that, on the average, Donovan bodies disappeared about 6 days after the initiation of therapy. Evidently, the response of the Donovan body to streptomycin is marked. The Donovan body does not fragment or decrease in gross size and appearance. The nuclear substance, however, rapidly loses its normal staining characteristics and appears to be wholly comprised of the remaining capsular material, which retains its normal form, size, and shape. Intracellular

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CHAPTER 36

GONORRHEA AND CHANCROID

GONORRHEA

Though penicillin is the undisputed therapeutic agent in the treatment of gonorrhea, resistant strains of gonococci have been produced *in vitro* by Miller and Bohnhoff (1), who showed that these strains were susceptible to streptomycin. Franks (2) reported four cases of gonorrhea that failed to respond to large doses of penicillin. Duemling and Horton (3) reported twenty-four cases of gonorrheal urethritis that revealed varying degrees of resistance to penicillin *in vitro*.

Some investigators doubt whether penicillin-resistant gonorrhea exists. In 2,821 cases of gonorrhea, Parkhurst, Harb, and Cannefax (4) did not encounter a single instance in which adequate amounts of penicillin failed to free the patient of the gonococcus. Spink (5) has never seen a gonococcus resistant to penicillin in adequate amounts.

If resistance to adequate dosage of penicillin should become a significant clinical problem, it would be encouraging to know, in advance, of the availability of another drug for treatment. Mortara and Saito (6) found that freshly isolated strains of *N. gonorrheae* were sensitive to streptomycin hydrochloride *in vitro* and were inhibited by 10 to 15 units per milliliter of medium. Putnam, Herwick, Taggart, and Chinn (7) initially treated four men having acute gonorrheal urethritis with 0.1 gm of streptomycin sulfate dissolved in 3 ml of physiologic salt solution at hourly intervals for five doses. Criteria of cure were three negative prostatic cultures within 10 days after treatment. All four patients were clinically and bacteriologically cured.

On the basis of this preliminary study, a larger series of cases was treated to determine the optimum dosage (8). Treatment with a single dose of 0.5 gm of streptomycin was first attempted and was found to give consistent cures. Table 77 shows the results of treatment with 0.5 gm and lesser amounts of streptomycin in water solution as a single injection into the gluteal muscle. All cases treated with a single injection of 0.3 gm or more

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0.5 to 1 gm a day. Therapy in such instances was prolonged, however, until healing took place, necessitating hospitalization for 20 to 60 days. Use of 4 gm a day for 5 days provided a rapid, relatively economical, and effective method of treatment without marked side reactions. With this regime, Donovan bodies disappeared from the lesions within 4 to 10 days, and healing usually took place 7 to 30 days after cessation of therapy.

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were cured. The failures on smaller doses were re-treated successfully by the following means: Two of the failures in the 0.1 gm group were cured by treatment with 300,000 units of penicillin in peanut oil and beeswax; the other failure in that group and the two failures in the 0.2 gm group were all cured by re-treatment with 0.2 gm of streptomycin.

This study was further extended (9) as reported in table 78. All failures occuring on 0.3 gm and the one failure on 0.5 gm were re-treated successfully with 0.6 gm of streptomycin.

Chancroid

Both the sulfonamides and penicillin have been found to be effective in the treatment of chancroid. Mortara and Saito (10) found that all strains of *H. ducreyi* which they tested were very sensitive to streptomycin. These authors used the drug successfully both prophylactically and therapeutically in experimental chancroid (11). Hirsh and Taggart (12) reported on the treatment of fifteen cases of chancroid with streptomycin.

The diagnosis was established on the basis of a positive culture inoculated with a swab of the lesion or pus aspirated from a bubo or by positive smears plus a positive Ducrey skin test. Fourteen patients were given 1.0 gm of streptomycin daily in divided doses intramuscularly every 4 hours. The fifteenth patient received 2 0 gm daily. Streptomycin was continued until the lesions showed evidence of complete healing. The duration of treatment ranged from 5 to 25 days. When there were large or multiple penile lesions, or lesions that were apposed or covered by skin, healing was slower and treatment was prolonged. Recovery was facilitated if the buboes were aspirated as they became distended. The histories of thirteen patients were followed for 1 to 8 weeks. The history of the patient who received 2.0 gm daily (24 gm in 12 days) was followed for 28 weeks after recovery. The patient who received the shortest course of treatment (5 days) had what was possibly a relapse 6 weeks after streptomycin was discontinued. This patient was successfully re-treated with sulfadiazine.

Later Taggart extended this study to include sixty-one cases of chancroid treated with streptomycin (table 79), forty-two of these patients being hospitalized, and nineteen treated on an ambulatory basis. Of the former, twenty-five were treated with divided doses every 4 hours, totalling 1 0 gm daily for 5 to 19 days until the lesions healed. The remaining seventeen were given 1.0 gm daily as a single injection for 5 days. All forty-two hospitalized patients were considered cured. The nineteen ambulatory patients were treated with a dose of 2 0 gm. of streptomycin as a single injection daily for 5 days. Of these patients, five were completely cured, six improved but had slight residual inguinal swelling, two improved but were lost from follow-up and six failed to obtain healing. One week after treatment

the lesions of these six patients had opened, had resumed their necrotic character, and were positive for *H. ducreyi* by Pappenheim's stain.

TABLE 77

Effect of streptomycin on gonorrhea (8)

NUMBER OF CASES	DOSE	NUMBER CURED	PERCENTAGE CURED
	gm.		
25	0.5	25	100
10	0 4	10	100
15	0.3	15	100
22	0.2	20	90 9
5	0.1	2	40.0

TABLE 78

Effect of streptomycin on gonorrhea (9)

NUMBER OF CASES	DOSE	NUMBER CURED	PERCENTAGE CURED
	gm.		
17	0 6	17	100
50	0.5	49	98
10	0.4	10	100
125	0.3	115	92
22	0 2	20	90 9
5	0 1	2	40

TABLE 79

Effect of streptomycin on chancroid

NUMBER OF CASES	TOTAL DAILY DOSE	METHOD	DURATION	NUMBER CURED	PERCENTAGE CURED
		Hospitalized patients			
	gm.		*days*		
25	1 0	Divided doses, 4-hour intervals.	5–19	25	100
17	1.0	Single injection	5	17	100
		Ambulatory patients			
19	2 0	Single injection	5	5	26 3

Healing was more rapid and progressed more favorably in the patients hospitalized than in those treated on an out-patient basis. The factor of bed rest was very important in influencing healing, although a few of the larger necrotizing ulcers in the hospitalized group received saline soaks for a short period of their hospital stay.

Although sulfonamides and penicillin appear to be the drugs of choice in the treatment of chancroid, streptomycin can be effective. For best results hospitalization appears to be indicated.

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Chapter 37

EAR INFECTIONS

The introduction of streptomycin for medical therapy of the ear presents the opportunity of treating certain refractory infections of the external auditory canal, middle ear, and mastoids with specific therapy. This new drug permits the rapid control of a high percentage of diseases of the ear for which we have had little to offer in the past, and as a result it is now possible to prevent many of the otogenic complications that were common a short time ago. Of less importance, but of great frequency, are some minor inflammatory diseases of the external ear, such as diffuse external otitis, which may now be controlled to a great extent by the topical use of streptomycin.

A therapeutic agent must come into intimate contact with the diseased areas to be fully effective. The anatomical configuration of the external auditory canal and the middle ear makes these areas relatively inaccessible to adequate local therapy except with special vehicles. Thus, because of their physiochemical properties and functions certain carriers have become popular for use in the topical application of medications to the external canal and middle ear. It has been shown by *in vitro* studies, however, that some of these vehicles appear to facilitate and others to prevent active diffusion of the contained antibiotic (1). When streptomycin was incorporated into water-soluble vehicles such as lanoline, "aquaphor," or "hydrosorb," little or no *in vitro* anti-bacterial action of the streptomycin was observed. On the other hand, effective *in vitro* antibacterial activity was obtained with water-soluble vehicles such as glycerin, "carbowax," and a mixture of "carbowax" and propylene glycol.

Aqueous solutions of streptomycin mixed readily with the ceruminous material formed in the external auditory canal Aqueous and low viscosity "carbowax" ear-drop preparations containing streptomycin may penetrate large perforations of the tympanic membrane and cover the surfaces of fenestration and radical mastoid cavities. They may also be instilled with a cannula into the middle ear by the otologist.

External Ear

It is necessary to differentiate the various the various forms of inflammatory diseases of the external ear and to define those categories in which

streptomycin may be of value (2). Diffuse inflammatory lesions of the external audity canal are due to gram-negative bacilli, gram-positive organisms, or mixed infections and occur mainly in tropical and subtropical areas. Symptoms range from mild dull aching to severe deep pain that may interfere with sleep. Physical findings consist of pain on manipulation of the auricle, slight to marked edema of the skin of the ear canal and periauricular tissues, and profuse sero-sanguinous to purulent-like drainage.

Fifty-four consecutive patients with findings that conformed to the above criteria for diffuse external otitis were examined and cultures taken (3). To compare the bacteriologic flora obtained from the ears of those patients with normal ears during the same season, cultures were made from the ear canals of twenty subjects (forty ears) without ear, nose, and throat complaints.

All infected ears showed gram-negative rods as the predominant organisms; by far the largest percentage of ears showed cultures of *Pseudomonas*. Occasional strains of *Alkaligenes*, *Aerobacter*, and *Proteus* were identified. Organisms isolated from the normal ears consisted of various strains of *Staphylococcus* and *Micrococcus* groups and an occasional nonhemolytic *Streptococcus*. With one exception, gram-negative rods were not obtained from normal ear canals

To evaluate the effects of topical therapy, groups of patients were treated with various concentrations of streptomycin in a "carbowax" ointment base, and the results of treatment were compared with those obtained in a similar group of patients treated with "carbowax" base alone.

The clinical results of therapy revealed that the two lower concentrations of streptomycin (0.25 mg/gm and 1 mg/gm of ointment) produced effects no better than those obtained from the use of the "carbowax" ointment base alone. Failure of treatment was not associated with pre-existing resistance of the organisms, since all of the isolated strains obtained prior to treatment were sensitive to concentrations of streptomycin far lower than those applied Treatment with 5 mg of streptomycin per gram of ointment gave results definitely superior to those obtained with the ointment alone Three patients in this series receiving intramuscular streptomycin failed to show any convincing improvement

The results of this study further indicated a high degree of correlation between clinical improvement and disappearance of *Pseudomonas* from the infected ear canal In general, a concentration of streptomycin that failed to produce a high percentage of cures failed also to produce a rapid disappearance of *Pseudomonas* in cultures taken from the treated ear On the other hand, the concentration of streptomycin found to be most effective (5 mg/gm) clinically resulted in rapid disappearance of *Pseudomonas* from such ears.

Senturia (4) reported sixteen additional cases which conformed to the above definition of acute diffuse external otitis. Thirteen of these patients received streptomycin in a "carbowax" ointment (5 to 10 mg/gm); all but two showed prompt subsidence of symptoms with rapid disappearance of discharge and of edema of the skin of the ear canal. Three patients were treated with aqueous solutions (5 mg/ml) in the form of ear drops. Improvement occurred in one, whereas pain and swelling increased in the other two.

Calloway (5) described one case of acute infectious eczematoid external otitis (diffuse type?) from which a pure culture of *Ps. aeruginosa* was obtained and which resisted all forms of therapy over a period of approximately 8 months. Streptomycin in aqueous solution (2.5 mg/ml) was instilled and sponged on the ear. The oozing and erythema subsided within 48 hours, the discharge stopped in 2 days, and cultures were negative in 11 days.

Pulaski and Matthews (6) reported five cases of chronic external otitis from which streptomycin-susceptible gram-negative bacilli and gram-positive cocci were cultured. The infections had been present 3.5 to 36 months, and a variety of antiseptics and chemotherapeutic agents had been used and found ineffective. The ear canals were flooded with a saline solution of streptomycin containing 5 to 10 mg/ml. This treatment was repeated once or twice daily or oftener when the drainage was copious. All patients responded rapidly with complete subsidence of drainage within 3 to 8 days.

No untoward reactions or sensitizations to the topical use of streptomycin were described by the above authors. There are, however, an increasing number of reports of sensitization (7, 8). Goldman and Feldman (9) described one white woman with otitis externa who was treated with streptomycin ointment (quantity and vehicle not stated). After a short period, the dermatitis of the ear canal flared up, and contact dermatitis developed on the skin of the eyelids, genitalia, arms, and buttocks. Reactions to patch tests with streptomycin were strongly positive

Middle Ear

No carefully controlled studies or reports of the effect of streptomycin on middle ear infections have come to our attention. This may be explained by the fact that almost all such infections yield to the sulfonamides or penicillin. Two groups of cases are available for study: (a) simple uncomplicated purulent otitis media, and (b) otitis media with associated diarrhea occurring in infants.

Twenty-one cases of otitis media treated with streptomycin have been reported by Terry (10). Routine treatment consisted of daily intramus-

cular injection of 2 to 3 gm in divided doses at 2-hour intervals and of local instillation into the ear (100 mg/ml) every hour throughout the waking hours. Twenty-six ears were treated and no other specific therapy was given. Thirteen ears showed cultures of *Proteus* and thirteen of *Pseudomonas*. There were complete drying in 50 per cent, definite improvement in 38 per cent, no improvement in 12 per cent. The response was as good with the *Proteus* as with the *Pseudomonas* group.

Pulaski and Matthews (6) reported thirteen cases of chronic otitis media treated with topical streptomycin. In most of the cases, mixed cultures of gram-positive organisms and gram-negative bacilli were obtained Some of the causative organisms were not subjected to sensitivity tests. Routine cleansing measures were employed, consisting of syringing out the ear canal and then flooding the cavity 1 to 3 times a day with streptomycin in sterile isotonic saline (5 mg/ml). Occasionally intratympanic instillations were performed.

In every case, the topical administration of streptomycin was followed by a change in the quantity and character of the discharge. Improvement in the appearance of the middle ear was general in all cases except one, and this was unexplained, since the organisms were susceptible. The suppuration ceased in six patients, whereas in two a minimal discharge persisted. One patient of particular interest was a 7-year-old child with chronic purulent otitis media, following scarlet fever, from which *S. viridans* was cultured. In spite of active treatment, the infection remained uncontrolled for 4 months. After the routine course of topical instillations of streptomycin, the drainage ceased and the infection remained clinically arrested.

Harris and Finland (11) reported findings on eighteen patients who had persistent purulent drainage from one or both ears and were treated with local instillations of streptomycin solutions Twenty-eight suppurating ears were treated, namely, six cases of subsiding acute otitis media with profuse purulent drainage, nineteen cases of chronic otitis media with intermittent or constant drainage over periods of 1 to 25 years; three ears with external otitis Cultures from twenty-six of the draining ears yielded gram-negative bacilli alone or (in five instances) mixed with gram-positive organisms. Almost all of these organisms were sensitive to streptomycin *in vitro*. *Ps. aeruginosa* and *Pr vulgaris* were the strains most frequently isolated.

Treatment as carried out by the patient consisted of drying out the ear and instilling 1 ml of streptomycin in saline (20 mg/ml) four times daily This was continued for 1 to 7 weeks The drainage disappeared during treatment in twelve ears and did not recur during 2 to 4 months of observation. In thirteen cases there was no apparent improvement, and drainage promptly recurred after streptomycin was discontinued. In three ears the

drainage subsided, but adequate follow-up was not available. Streptomycin resistance and a change of bacterial flora were observed frequently. The authors concluded that the results were not uniformly favorable, although they noted that simple local instillation of streptomycin did benefit some of the refractory cases.

Walsh and Stone (12) presented a preliminary report on the use of streptomycin in otitis media which occurred in seven infants and children. These included four chronic suppurative cases, two cases of acute otis media with associated diarrhea, and one with associated bronchiectasis which did not respond to penicillin and sulfadiazine therapy Cultures of *Pseudomonas*, *E. coli* and *S. albus* were obtained from the ears. Normal saline, peroxide, and 2 per cent acetic acid were used for irrigating the external and middle ear. Streptomycin was then given intramuscularly in doses of 50 to 100 mg every 3 hours with excellent control of the infection and improvement in the associated disease. No toxic effects were noted. These authors conclude that streptomycin appeared to be very effective in acute middle ear infections particularly when *Pseudomonas* was the causative organism.

Senturia and Ingram[1] (13) reported thirty-seven unselected infants and children who entered the hospital for treatment of otitis media, antritis, mastoiditis, and associated diseases. In a high percentage of the cases, cultures were obtained after institution of chemotherapeutic or antibiotic therapy, and there was no bacterial growth on cultures. Streptomycin-sensitivity tests were available in a small number of the cases.

It was the policy of the staff to treat these critically sick patients with a combination of sulfonamides (approximately 0.2 gm/kilo/24 hours), penicillin (approximately 30,000 units every 3 hours) and streptomycin (approximately 50 mg/kilo/24 hours). As quickly as positive culture reports were obtained and sensitivity of the organisms was determined, or as soon as the clinical condition of the patient permitted, the superfluous drugs were discontinued

Consideration of the nature of these clinical data makes it obvious that positive conclusions, in respect to streptomycin, cannot be drawn from this study Certain definite impressions and negative findings may be of distinct value, however, and do offer some information on this problem about which so few clinical or experimental data are available.

Twelve cases were placed in the category of severe acute or recurrent suppurative otitis media. Where reported, cultures showed *Pseudomonas* or *S. aureus* or *albus*. Ten of these cases showed prompt improvement, whereas in two cases symptoms failed to subside after 4 days of streptomy-

[1] The authors are indebted to Dr Alexis F. Hartmann, Physician-in-Chief of St Louis Children's Hospital, for assistance in this study.

cin therapy and the drug was discontinued. In sharp contrast to this group are five cases of suppurative otitis media with associated diarrhea treated in the same manner. Three of these cases were unimproved; one case was placed in the uncertain category; and one patient showed slow but progressive improvement on streptomycin therapy. It would appear from these data that streptomycin combined with penicillin and sulfonamides was effective in controlling uncomplicated suppurative otitis media. The inclusion of this antibiotic in the therapy of otitis media with associated diarrhea was not productive of equally good results.

At the Fourth Streptomycin Conference under the auspices of the Veterans Administration (14) it was reported that streptomycin was used locally in the treatment of twelve cases of tuberculous otitis media, of which six were reported healed and six improved. The minutes of the Fifth Conference (15) state that thirteen cases of otitis media were treated. Drainage ceased in eleven, and in six of these the perforations healed. It was assumed that diagnosis was established in all cases by positive culture of the drainage material.

Mastoid

Pulaski and Matthews (6) reported one case of uncomplicated otitis media and mastoiditis. Cultures revealed *Pr. vulgaris* which was sensitive to streptomycin. Streptomycin was given topically and intramuscularly (0.125 gm every 3 hours) and a radical mastoidectomy was performed. A rapid uncomplicated convalescence followed.

Senturia and Ingram (13) reported eleven cases of acute antritis or mastoiditis occurring in children, five of whom showed rapid improvement on combined therapy (penicillin, sulfonamides, and streptomycin). Five patients required surgical drainage of the mastoid, four of whom responded promptly while the other continued to show a discharging ear. The eleventh patient was overwhelmed by the infection and died in 5 days despite streptomycin and penicillin treatment. Results from combined therapy were so satisfactory that surgical intervention was avoided in approximately half of these cases. This is in contrast to a group of six small children with otitis media, antritis, and associated diarrhea. This latter group did not respond to combined therapy (penicillin, sulfonamides, and streptomycin), and their condition went rapidly downhill until a critical state was reached Because of the progressive clinical deterioration of these patients, mastoid antrotomy was performed in each case with prompt and startling improvement in the clinical course and eventual cure of the ear infection and associated diarrhea

These authors conclude from their data that response to combined therapy was excellent in uncomplicated severe acute infections of the middle

ear and mastoid, suggesting that there may be definite advantage to this form of therapy in the more severe infections appearing in the children. No immediate evidence of local or general toxicity resulting from the use of streptomycin was observed in this series. This may be explained on the basis of relatively low dosage, inherent drug tolerance in children, and limited period of treatment with streptomycin. A small number of patients with antritis and associated diarrhea who failed to respond to penicillin, sulfonamide, and streptomycin therapy showed prompt and striking improvement as soon as surgical drainage of the middle ear, antrum, or mastoid was performed.

Fowler (16) packed a mixture of streptomycin (0.2 to 1 mg/ml) and sulfamylon (5 per cent) into radical mastoidectomy cavities, keeping the inner wick saturated with the solution until removed 1 week postoperatively. Drops of this mixture in a "carbowax" or methyl cellulose (1 per cent) vehicle were instilled for several days. This author believed that the cavities dried more promptly on this regime.

Two infants with tuberculous involvement of the middle ear and mastoid were reported by Senturia and Ingram (13). A 19-month infant with tuberculous otitis media and spreading lung infection received intramuscular streptomycin (1 gm/day) for approximately 2 months. After 1 month the ears were dry and healed, and adenoidectomy was performed uneventfully The second case, an infant of 2 months, was seen with bilateral otorrhea of at least 2 weeks' duration, a positive tuberculin (1:100,000), and a culture of *M. tuberculosis* from the ear discharge. Under combined therapy (including 400 mg of streptomycin daily) there was less ear discharge for a time, but granulations rapidly filled the ear canals, and the patient expired after approximately 2 months of observation. In view of the results reported above, surgical exenteration of the diseased bone of the mastoid, while under streptomycin, might have prevented the fatal result of this case

One adult male of 30 years was observed by Senturia and Walsh (17) with tuberculous otitis media, mastoiditis, and a postauricular fistula persisting after radical mastoidectomy. One gram of streptomycin was given intramuscularly in five divided doses, and later twice daily for a little more than 2 months. A saline solution containing 1 gm of streptomycin was used to irrigate the ear canal and for application to the fistula region twice daily for approximately 6 weeks. The fistula was healed over after 3 weeks of treatment, and the middle ear became dry after 6 weeks. There has been no recurrence while under observation for 1 year following discharge from the hospital.

The prompt response of the tuberculous fistula was anticipated as a result of the reports of the Veterans Administration. At the Fourth Con-

cin therapy and the drug was discontinued. In sharp contrast to this group are five cases of suppurative otitis media with associated diarrhea treated in the same manner. Three of these cases were unimproved; one case was placed in the uncertain category; and one patient showed slow but progressive improvement on streptomycin therapy. It would appear from these data that streptomycin combined with penicillin and sulfonamides was effective in controlling uncomplicated suppurative otitis media. The inclusion of this antibiotic in the therapy of otitis media with associated diarrhea was not productive of equally good results.

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was discontinued because of the marked resistance of the organism. Radical mastoidectomy and ligation of the jugular vein were performed, and the patient made an uneventful recovery on penicillin and triple sulfonamide therapy.

This case presents the problem that may arise from the indiscriminate use of streptomycin in a patient with an undrained infected mastoid. Rapid streptomycin tolerance undoubtedly developed, and when surgery was performed, streptomycin was not available for coverage of the gram-negative bacillus infection.

Summary

The data indicate that streptomycin offers tremendous help in the control of selected ear infections, that it allows safer surgical intervention, and that it provides a more uneventful postoperative course in those diseases of the ear which are not readily controlled by the sulfonamides and penicillin.

When streptomycin, penicillin, and sulfonamides are given to the critically ill child, a combined coverage for both gram-positive and gram-negative organisms is provided. Control of the disease process is frequently obtained long before an etiologic diagnosis is made. When the direct smear or culture report is received, the superfluous drugs may be discontinued. In this manner, the disease may be controlled rapidly and effectively, and, at least in children, few toxic reactions are noted.

It has been the experience of most of the writers that streptomycin treatment or combined therapy serves only as a temporary control of the surgical diseases of the ear. It is essential that suppurative infections of the middle ear, antrum, or mastoid receive surgical drainage, and where indicated, therapy should be continued postoperatively. Rapid streptomycin resistance develops in cases where medical therapy is provided without surgical drainage

In a large number of the reported cases, good results have been obtained from the topical use of streptomycin. Diffuse external otitis and uncomplicated middle ear infections with large tympanic perforations should respond to this form of therapy. Whenever possible, it would appear desirable to combine topical with intramuscular therapy in ear diseases which involve deep-seated infections

Formerly, the mortality among infants with otitis media, antritis, and associated diarrhea was very high In carefully selected cases the combination of surgical drainage and streptomycin therapy should offer a high percentage of cures.

The preliminary results with streptomycin in tuberculous otitis media and mastoiditis offer promise of controlling the early lesions and provide the

ference (14) a series of patients with a total of 195 draining sinuses were reported. Of these, 118 were healed, fifty-one improved, and twenty-six were unchanged after a daily regimen of either 1.0 or 2.0 gm of streptomycin. At the Fifth Conference (15), 100 cases with sinuses originating in bone or cartilage received daily doses of 2, 1, or 0.2 gm of streptomycin In each group, with one or two exceptions, healing occurred and there was no recurrence.

The use of streptomycin in tuberculosis of the middle ear and mastoid is still to be evaluated. It appears, however, that streptomycin halts the extension of the destructive process. Of equal importance is the consideration that streptomycin will allow surgical exenteration of the involved mastoid bone where previously surgical intervention was considered too dangerous and would permit unrestricted spread of the disease.

Otogenic Complications

Pulaski and Matthews (6) reported two cases of otogenic complications. The first was that of meningitis, osteomyelitis, and brain abscess secondary to otitis media and mastoiditis, from which a culture of *S. aureus* (coagulose positive) was obtained. The second was that of mastoiditis with complicating lateral sinus thrombosis and meningitis, from which hemolytic *S. aureus* and hemolytic streptococci were grown.

In Case I, streptomycin was given intramuscularly in dosages of 3.2 gm daily with only slight improvement Remarkable progress occurred after the daily intrathecal use of 0.25 gm of streptomycin. A total of 58 gm of streptomycin was given over a period of 19 days Case II failed to respond to penicillin, and streptomycin was given (0.25 gm intramuscularly every 3 hours). Immediate clinical improvement occurred, but meningitis and lateral sinus thrombus developed. After surgical intervention the patient made an uneventful recovery

Senturia and Ingram (13) described a 2-week-old premature infant who entered the hospital with dehydration and diarrhea and developed otitis media and meningitis Ear cultures yielded staphylococci, a perianal abscess grew *E coli*, and the spinal fluid gave a negative culture The patient did not improve on sulfonamides and penicillin therapy, but did respond promptly to streptomycin treatment

Senturia and Walsh (17) described a 11-year-old white female with a discharging right ear of 2 years' duration who developed chills and fever The local physician gave penicillin and streptomycin (dosage not available) with partial control of the symptoms When the patient was seen there was evidence of otitis media, mastoiditis, and lateral sinus thrombosis. Cultures from the ear and mastoid antrum revealed *E coli*, which was not inhibited by 0.05 mg of streptomycin After 4 days, streptomycin therapy

was discontinued because of the marked resistance of the organism. Radical mastoidectomy and ligation of the jugular vein were performed, and the patient made an uneventful recovery on penicillin and triple sulfonamide therapy.

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In Case I, streptomycin was given intramuscularly in dosages of 3.2 gm daily with only slight improvement. Remarkable progress occurred after the daily intrathecal use of 0.25 gm of streptomycin. A total of 58 gm of streptomycin was given over a period of 19 days. Case II failed to respond to penicillin, and streptomycin was given (0.25 gm intramuscularly every 3 hours). Immediate clinical improvement occurred, but meningitis and lateral sinus thrombus developed. After surgical intervention the patient made an uneventful recovery.

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CHAPTER 38
OPHTHALMOLOGY

The principles of streptomycin therapy in ocular infections are no different from those in other types of infections. Two requirements must be met, that is, the organisms must be sensitive to the action of streptomycin, and the streptomycin must reach the area of the infection. To understand the reasons for the limitations of streptomycin therapy in ocular infections, a knowledge of the anatomical structures concerned is needed.

For our purpose the eye and its adnexa may be divided into two parts: the first consists of the vascular tissues, and the second consists of avascular tissues along with the intraocular fluids. The lids, extraocular muscles, lacrimal apparatus, sclera, and uveal tissues belong to the first group; the cornea, aqueous humor, vitreous humor, and lens belong to the second group.

The cerebral layers of the retina contain its blood supply, but, like the central nervous system, the glial sheaths surrounding the blood vessels keep out many of the constituents found in the blood stream.

The tissues of the first group receive their nutrients directly from the blood and are similar to other tissues of the body in response to infection and in the concentration of the various substances circulating in the blood stream. The tissues and fluids in the second group differ from the first in their response to infections and in the concentration of the various constituents of the serum. The latter concentrations depend upon highly selected filtrate processes by which the aqueous is formed.

DISTRIBUTION OF STREPTOMYCIN IN THE EYE

From these preliminary considerations, it is not surprising that, following the systemic administration of streptomycin, the concentration of this agent differs in the various ocular tissues and fluids. It has been demonstrated that when rabbits received a single intravenous or intramuscular injection of 10,000 μg/kg body weight, streptomycin was detected in the conjunctiva, sclera, extraocular muscles, and aqueous humor (1). For streptomycin to become detectable in the cornea, vitreous, chorioretinal tissues, and optic nerve, *massive injections of 100,000 μg/kg were required.*

opportunity of performing major surgical procedures without danger of fatal otogenic complications.

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immediate inflammatory reaction following the application of buffered solutions of streptomycin. Bellows and Farmer (2) found that the present commercial streptomycin (hydrochloride form) as well as the highly purified streptomycin-calcium-chloride complex in concentrations of 10,000 μg/ml of saline solution instilled into human and rabbit eyes, did not produce a greater degree of conjunctival redness or smarting than that occurring upon similar treatment with accepted ophthalmic solutions.

In addition to the production of conjunctival redness and fluorescein-staining reaction of the cornea, another means of evaluating local toxicity of a drug is its influence upon the regeneration of the corneal epithelium. As shown by Bellows (5), the cornea is an excellent medium to ascertain information relative to the local tissue effect of drugs. Bellows and Farmer (2) denuded the cornea of its epithelium and applied streptomycin in saline solution five times daily. It was found that the concentrations of 10,000 μg/ml of saline solution did not retard the normal process of corneal regeneration but solutions containing 50,000 μg/ml of saline solution not only delayed the healing but also caused marked vascularization and scar formation of the cornea.

Intraocular Injection of Streptomycin

In a preceding section it was stated that even after massive doses of streptomycin were given systemically, barely detectable quantities were found in the vitreous. It followed naturally for investigators to determine whether the direct injection of streptomycin into the vitreous would be a practical method of administration. Bellows and Farmer (6) employed solutions of various commercial lots of streptomycin and a highly purified sample of streptomycin-calcium-chloride complex in concentrations ranging from 250 to 10,000 μg/ml prepared in saline.

The respective solutions in doses of 0.1 ml were injected with a 27-gauge needle, the puncture being made at the equator of the eyeball. The frequency and number of permanent vitreous opacities were low with the more purified commercial samples (twenty-one eyes—six with opacities) and least with the highly purified streptomycin-calcium-chloride complex (nineteen eyes—four eyes with opacities). An occasional lens opacity appeared, which was attributable to trauma by the needle or to injection of the saline too close to the lens. Leopold, Wiley, and Dennis (3) confirmed these findings by reporting that the direct intravitreal injections in concentrations below 800 μg produced minimal and limited damage to the globe. From these observations it may be asserted that any theoretic objection to intravitreal injections of streptomycin in certain cases of severe purulent ophthalmia, which, treated by older methods would invariably lead to blindness, are unfounded Bellows and Farmer (6) found that following

Such massive injections cannot be recommended for man because they might readily lead to neurotoxic symptoms, particularly if employed for a prolonged period.

Penetration through the Cornea

Because of the aforementioned disadvantages of systemic administration of streptomycin, investigations were undertaken to determine whether adequate therapeutic concentrations of streptomycin could be achieved by local methods of administration.

It was soon found that streptomycin does not penetrate through the normal cornea; however, satisfactory concentrations of streptomycin in the aqueous humor are readily obtained if a wetting agent or iontophoresis is employed. Furthermore, if the cornea is abraded or infected, streptomycin penetrates the cornea readily. Leopold and Nichols (1), using ion-transfer and a solution containing 5,000 μg/ml of streptomycin in saline, found from 30 to 70 μg/ml of streptomycin in rabbit's aqueous. Other investigators (2) found 25 μg in aqueous of rabbits when 10,000 μg was employed with ion-transfer. It is also possible to obtain a high concentration of streptomycin in the vitreous by means of ion-transfer if streptomycin is injected retrobulbarly. By this means Leopold, Wiley, and Dennis (3) reported values up to 15 μg/ml of vitreous.

Bellows and Farmer used as a wetting agent one drop of aerosol (O.T.)

50,000 μg/ml for 30 minutes, 1 hour, and 2 hours. Assays of the aspirated aqueous revealed values of 25 to 50 μg/ml when the last concentration was used. With 10,000 μg/ml, the values were below 6.25 μg/ml aqueous for periods less than 1 hour. After 2 hours, however, a concentration of 25 μg/ml of aqueous was found.

In animals with abraded corneas, 20 to 21 μg/ml streptomycin in aqueous was found 15 minutes after drop instillations of 50,000 μg/ml of isotonic solutions (1). Under similar circumstances a corneal bath with a saline solution containing 10,000 or more μg/ml for 2 hours may yield concentrations of more than 100 μg/ml of streptomycin in aqueous. In rabbits with vaccinia keratitis a corneal bath containing 10,000 μg/ml streptomycin in saline solution for 15–30–60-minute periods produced aqueous concentrations between 25 and 200 μg/ml (2). Apparently no relation exists between the longer and shorter intervals of bathing and the assay values. The latter seemed to depend rather upon the size of the corneal lesion.

Local Effects of Streptomycin

Streptomycin applied to the human eye is well tolerated. With earlier samples, however, Mohtor (4) reported that the conjunctiva presented an

ever, to confine the infection to the vitreous humor. Direct intravitreal injection of 25 to 100 μg of streptomycin prevented infections if administered within 6 to 8 hours after inoculation. Similar findings were reported by Leopold, Wiley, and Dennis (3), who demonstrated that the direct intravitreal injection of streptomycin reduced the severity of experimental vitreous infections with *E. coli*. These investigators found that retrobulbar injections of streptomycin plus iontophoresis were more effective than anterior chamber injections in combating vitreous infections.

Streptomycin in experimental ocular tuberculosis

The brilliant investigations of Feldman, Hinshaw, and Mann (7,8) definitely established the marked deterrent and therapeutic effect of streptomycin in experimental guinea pig tuberculosis. It is not surprising, therefore, that ophthalmologists (9, 10, 11) became interested in the possibilities of streptomycin therapy in experimental and clinical ocular tuberculosis.

Bietti (9) showed that streptomycin was of value in experimental ocular tuberculosis infection in rabbits if the treatment was begun early with streptomycin alone or in a combination with promin If treatment was instituted after appearance of the ocular lesions, however, the infection was not inhibited. Furthermore, he noted that even in animals treated early, the tuberculous process developed when the streptomycin administration was stopped. Somewhat similar findings were reported by Grignolo (10). This investigator noted marked inhibition of the tuberculous process in guinea pigs receiving streptomycin therapy commencing 6 days after the inoculation. If treatment was begun after 15 days, no significant difference in the progress of the infection was noted in the group of guinea pigs receiving treatment and those in the control group.

Woods (11) inoculated the anterior chambers of so-called immune- allergic rabbits with a virulent strain of tubercle bacilli. The ocular lesion of the animals became inactive after 8 weeks of streptomycin treatment Histologic examination, however, disclosed minimal to moderate degrees of tuberculous activity, and transmission experiments disclosed that three out of six animals had positive infections of the uvea Upon cessation of treatment, two of eight rabbits developed an active disease process. The results of streptomycin therapy were enhanced when promizole was employed at the same time, but even this combination did not possess complete bactericidal action against tubercle bacilli

Streptomycin Therapy in Clinical Ocular Infections

Indications

Clinical reports on use of streptomycin therapy in ocular infections are very sparse. From theoretical considerations, it may be expected that

the injection of 100 μg of streptomycin into the vitreous, 12.5 to 25 μg of streptomycin was present even 24 hours after the injection.

Streptomycin Therapy in Experimental Ocular Infections

Ps. aeruginosa infection of cornea

Infection of the cornea with *Ps. aeruginosa* usually produces a severe reaction, frequently leading to destruction of the cornea. The advent of sulfonamide therapy greatly reduced the severity of infection; and following experimental inoculation, sulfonamides, if applied early enough, would often prevent infection. Bellows and Farmer (6), employing a virulent strain of *B. pyocyaneus* which was sensitive to streptomycin, were able to prevent infection of the cornea when the antibiotic was applied within 6 hours after inoculation of the rabbit's cornea.

Vaccinia keratitis

Streptomycin, when applied locally to rabbit's corneas after inoculation with vaccinia, did not prevent the resulting keratitis (6). However, the number of organisms responsible for the accompanying secondary infection was greatly reduced in the treated eyes. As a result of this, there was less scarring and vascularization of the corneas of treated eyes.

Streptomycin therapy of vitreous infections

The vitreous of rabbits were inoculated with 1,000 to 2,000 organisms from a 24-hour broth culture of *S. pyogenes* strain C 203 by Bellows and Farmer (6). The animals were separated into three groups and treated by (a) continuous intravenous drip of 100,000 μg of streptomycin solution per kilogram of body weight over a period of 6 hours; (b) intramuscular injections of a similar amount of the antibiotic emulsified in a sterile preparation of cholesterol derivatives in peanut oil with 2 per cent beeswax (pendil improved); (C) intravitreal injections of 250 to 1,000 μg of streptomycin in 0.1 cc of an isotonic solution of sodium chloride. None of these forms of systemic administration was effective in preventing vitreous infections, but all of them prevented spread of the infection beyond the vitreous, whereas in untreated animals the external manifestations of the infection were much more pronounced. This result is easy to understand if one considers the fact that the concentration of streptomycin is but 2 μg/gm of vitreous following a single intramuscular injection of 100,000 μg of streptomycin per kilogram of body weight (1). Since the *in vitro* sensitivity of the organism employed by Bellows and Farmer (6) was 10 μg/ml, the concentration of streptomycin in the vitreous was inadequate for this organism, and infection resulted The concentrations in the conjunctiva, sclera, extraocular muscles, and aqueous humor were sufficiently high, how-

ever, to confine the infection to the vitreous humor. Direct intravitreal injection of 25 to 100 μg of streptomycin prevented infections if administered within 6 to 8 hours after inoculation. Similar findings were reported by Leopold, Wiley, and Dennis (3), who demonstrated that the direct intravitreal injection of streptomycin reduced the severity of experimental vitreous infections with *E. coli*. These investigators found that retrobulbar injections of streptomycin plus iontophoresis were more effective than anterior chamber injections in combating vitreous infections.

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Streptomycin Therapy in Clinical Ocular Infections

Indications

Clinical reports on use of streptomycin therapy in ocular infections are very sparse. From theoretical considerations, it may be expected that

streptomycin will be of slight or no value in certain intraocular inflammatory diseases such as iridocyclitis or iritis, conditions in which the etiology is often obscure or is of noninfectious nature Futhermore, even in those instances where the intraocular inflammation should prove to be of bacterial origin, the problem of achieving an adequate therapeutic concentration without producing neurotoxic symptoms must be overcome. Systemic streptomycin therapy, either alone or in combinations with other agents, may be tried, however, in ocular involvement with tuberculosis, tularemia, brucellosis, and lymphogranuloma venereum. Impending or early purulent ophthalmia following intraocular surgery or penetrating wounds requires the administration of streptomycin combined with penicillin by means of ion-transfer or by direct injections into the eyeball. Except in the aforementioned diseases, use of streptomycin in ocular infections must be confined to its local application in serious external ocular infections caused by agents sensitive to the action of streptomycin. For this purpose, streptomycin may be applied in a concentration of 10,000 μg/ml of saline solution or per gram of ointment base. The application should be made at 3-hour intervals, or more often if the infection is very severe. Streptomycin should not be used indiscriminately in self-limited or mild forms of conjunctivitis because of the possibility of the development of hypersensitization needlessly in susceptible persons.

Acute conjunctivitis

Streptomycin has been shown to be of value in certain types of acute conjunctivitis (6). In one case of purulent conjunctivitis caused by *N intracellularis* Group IIa, a marked improvement was noted in 24 hours In a second case of acute conjunctivitis produced by *B. subtilis*, improvement occurred within 48 hours In a third patient with acute conjunctivitis in which a pure culture of diptheroids was found, improvement was noted within 3 days In three other patients in which there was a mixed infection and in one case of acute conjunctivitis in which organisms were not found, there was a favorable response to streptomycin therapy. Grignolo (12) reported good results with streptomycin in cases of acute staphylococcus and Koch-Weeks conjunctivitis. Complete recovery in 1 to 5 days occurred when four patients with gonorrheal conjunctivitis, fifteen with Koch-Weeks, and twenty-nine with Morax-Axenfeld conjunctivitis were treated with instillations of streptomycin in a concentration of 10,000 μg/ml of solution (13) Streptomycin was found to be ineffective in epidemic keratoconjunctivitis (13, 14)

Chronic conjunctivitis

The effect of streptomycin therapy in chronic forms of conjunctivitis is about equal to that of other agents employed for these conditions (6).

Where organisms were found, and in particular if sensitivity tests revealed the organisms to be sensitive to streptomycin, the conjunctival sacs became sterile when streptomycin was employed. In spite of this, the clinical appearance frequently would remain unchanged, suggesting that other factors, such as chronic meibomitis, were active in continuing the conjunctival inflammation.

Trachoma and inclusion conjunctivitis

Bellows and Farmer (6) found that streptomycin exerted a favorable effect upon one patient showing the clinical characteristics of trachoma. In twelve cases of florid trachoma treated with streptomycin by Grignolo (12), the conjunctival sacs became sterile, but the nodules were uninfluenced. In one case of inclusion conjunctivitis that received streptomycin, inclusion bodies disappeared rapidly (12).

Blepharitis

Good results were obtained in the ulcerative form of blepharitis treated with streptomycin (12). The author, however, has found local application of streptomycin to be of slight value in most forms of blepharitis (14).

Corneal infections

Streptomycin appears to be of value in certain forms of corneal infections In one case of severe corneal infection caused by *E coli*, a satisfactory response was obtained following local application of streptomycin (15). Other investigators also found this form of therapy effective in other forms of corneal ulcers (6, 16). On the other hand, Grignolo noted no improvement in corneal infections produced by pneumococcus, staphylococcus, or streptococcus (12). In phlyctenular keratitis, streptomycin was found to be ineffective (12, 14).

Panophthalmitis

In one case of panophthalmitis in which a pure culture of *Pr. vulgaris* was found, Somerville-Large (17) reported a favorable result with streptomycin although the infection did not respond in time to prevent perforation. Still, the cultures became negative with streptomycin. Earlier treatment with sulfadiazine and penicillin had been ineffective. From the experimental evidence mentioned earlier, total destruction of the globe might have been prevented if streptomycin had been injected intraocularly early in the course of this disease. In purulent ophthalmia in which the offending organism proves to be sensitive to streptomycin, one is justified in directly injecting streptomycin intraocularly, otherwise the eye inevitably becomes blind.

Lymphogranuloma venereum ophthalmia and brucellosis

So far no report has been made on the use of streptomycin in infections of the eye with lymphogranuloma venereum or brucellosis. One would be justified, however, in employing streptomycin alone or in combination with other agents in such instances.

Oculoglandular tularemia

Heilman (18) showed that experimentally produced tularemia responded favorably to streptomycin. In nonoculoglandular tularemia, Foshay and Pasternack (19) and others have reported good results. In one case of oculoglandular tularemia, Minden and Springer (20) reported that an immediate and dramatic improvement was produced by streptomycin, though they admit that a low-grade fever persisted for 30 days.

Tuberculous disease of the eye

The great difficulty in establishing a diagnosis of intraocular tuberculous infection makes it a difficult task to evaluate the effect of streptomycin in this disease. In the small group of cases reported thus far, the results are promising and warrant further investigation of this form of therapy.

In three cases of tuberculous disease of the choroid, Grignolo reported a favorable response to streptomycin therapy. In eight cases of ocular tuberculosis included in the report by the Streptomycin Committee of the Veterans Administration (21), there was some degree of improvement in three, complete healing in two, and no change in three.

Even better clinical results may be expected from a combination of streptomycin with other agents such as promin and promizole. Futhermore, it is likely that these drugs will prove to be particularly useful when employed prophylactically to prevent relighting the infection in quiescent tuberculous eyes undergoing surgical procedures such as cataract extraction

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CHAPTER 39
SKIN INFECTIONS

Little has been written about the use of streptomycin topically in cutaneous diseases. This probably is due to the fear of sensitization reactions inculcated by untoward experiences with such reactions in topical penicillin therapy.

Several diseases are relegated to this section. Some of these are clearly defined entities with clear-cut etiology, such as lupus vulgaris, impetigo, and pustular folliculitis; others, such as infectious eczematoid dermatitis, may well have a double etiology: a primary contact eczema may, for example, become secondarily infected with staphylococcus or other bacteria.

In general, proper treatment of skin diseases with antibiotics differs in no wise from the ideal procedures and goals set in other branches of medicine.

The specific causative organism should be determined where possible, and the particular drug selected according to the sensitivity of the organism to it, and in terms of the sensitizing potentialities of the drug in a particular case. In cases caused by organisms sensitive to streptomycin, immediate benefit from use of the drug is observed. In general, these cases include the pyodermas due to gram-positive organisms, stasis ulcers, and dermatophytosis secondarily infected with gram-positive and sensitive gram-negative organisms

The means of administration depends upon the type and extent of the disease to be treated If the cutaneous disease is extensive or essentially of systemic character, parenteral administration is indicated according to schedules and dosages outlined elsewhere by other authors in this book. If the disease is of limited extent and more strictly of cutaneous nature, it should be treated topically, with the drug included in either a grease cream or a gel base, in concentrations of about 5,000 units/gm (1, 2). Prior to application of the ointment, the skin should be thoroughly cleaned with soap and water and all the crusts, scales, and other detritus removed. Streptomycin ointment should be applied at least four times a day.

The physician should be aware of the possibility of inducing cutaneous sensitization through application of streptomycin to the skin. Goldman

and Feldman (1) have recorded a 1 per cent occurrence of sensitivity following patch testing with ointment mixtures containing 5,000 units/gm, but they do not consider the hazard too great. Observations of Strauss and Warring (2, 3) and others (4, 5, 6, 7, 8, 9) as well as those of the writer (10) indicate, however, that continued exposure of patients or of individuals handling the drug may increase the number of sensitizations to a marked degree (50 per cent or more) and these may be of very severe proportions. It would appear that patch testing does not give a reliable indication of the potentialities for sensitization. The possibility of development of severe eczematous reactions upon exposure of the skin directly to streptomycin must be kept in mind. It is hoped that reactions to cutaneous contact will not reach the proportions that have been found to occur with cutaneous penicillin therapy (13).

The possibility of secondary sensitizations produced by exposure to other sensitizing agents in patients already sensitized to one must be guarded against (12).

Very limited experience with dihydrostreptomycin indicates that comparable skin sensitization may occur with this form of the drug, and that patients already sensitized to streptomycin may have severe cutaneous reactions to dihydrostreptomycin. Hobson *et al.* (14) have indicated that the dihydro form may be used parenterally in some cases sensitive to streptomycin. The work of Edison *et al.* (15) has shown that a similar antibacterial spectrum is to be expected for both forms of the drug and that organisms resistant to streptomycin will likewise be resistant to the dihydro form. Indications are, however, that toxicity is less with dihydrostreptomycin.

Specific diseases, such as granuloma inguinale, gonorrhea, and chancroid, which conceivably might be considered here, are dealt with extensively elsewhere in this volume.

The following isolated accounts on various skin diseases may well be considered.

O'Leary *et al* (16), studying cutaneous tuberculosis, obtained encouraging results by parenteral use of streptomycin. Healing of open ulcerative lesions occurred, but the granulomatous process was still evident in the healed areas of the lesions. These investigators felt that complete cure was not obtained.

Cornbleet (17), who combined calciferol and streptomycin in the treatment of five cases of lupus vulgaris reported that lesions became inactive in 6 to 9 weeks. This author suggested synergistic action between these two drugs. Futher studies are needed.

Closure of tuberculous ulcers of the tongue was recorded by Shamaskin (18) and by Wolfer *et al.* (19)

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Costigan (20) and Jacobson and Cloward (21) reported cases of actinomycosis cured with streptomycin. Costigan used 250,000 units parenterally every 3 hours for 5 days with no other therapy. Jacobson combined streptomycin with penicillin and sulfonamides. Dunham and Rake (22) demonstrated that penicillin G is approximately 3,000 times as effective as streptomycin against the spirochete of syphilis.

Paget (23) considers the value of streptomycin in treatment of leprosy to be still unsettled, and Cuttle (24) reported failure in one case. The problems raised by the toxicity of the drug during long-continued use are as evident here as in the treatment of tuberculosis.

The pathogenic fungi other than Actinomyces and Nocardia are, in general, not sensitive to streptomycin (25). Therapeutically, therefore, streptomycin is of no value against most fungal infections. From the diagnostic point of view, however, both penicillin and streptomycin are extremely useful. When added to culture media used in the isolation of pathogenic fungi, they suppress sensitive bacteria and permit easy isolation in relatively pure culture (26). For practical purposes, a concentration of 25 units of each drug per milliliter of medium can be used Addition of oxgall and crystal violet (1:100,000) to the medium containing the penicillin and streptomycin markedly retards growth of saprophytic fungi also, thus furthering isolation procedures (27, 28).

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CHAPTER 40

TOXICITY OF STREPTOMYCIN IN THE HUMAN

Streptomycin is a reasonably safe antimicrobial agent when administered to the human. In recommended dosage schedules, with wise clinical observation and with sufficient laboratory tests, it can be given without fear of sudden catastrophe. There are, however, definite untoward reactions which prevent its widespread and indiscriminate prescription to the human. These are negligible in number and severity with parenteral administration of no more than 4 gm daily for periods of less than 7 days. When longer administration of streptomycin is contemplated, multiple laboratory observations are recommended, to be performed concomitantly throughout the duration of therapy, because its extended use in the human is accompanied by a calculated risk of significant proportions from toxic reactions. It is an established fact that most individuals receiving streptomycin for longer than 1 week will demonstrate one or more of these reactions.

The incidence of reactions increases in direct relation to total daily dose, a striking increase occurring with doses of more than 1 gm daily (fig 86 and table 80) The data are taken (*with few modifications*) directly from a study of tuberculosis made cooperatively by the Army, Navy, and Veterans Administration (1) With daily doses of 1.8 to 2 gm for 60 to 120 days, there is an appreciable increase in the incidence of toxic reactions in every category over those experienced with the administration of 1 gm With 0 5 gm daily, there is a further decrease in number of most reactions The reduced frequency of toxic reactions following the administration of 1 gm daily in two, rather than five, divided doses is difficult of interpretation. In the latter case (five doses) there will be maintained, in the serum of patients, constant concentrations of streptomycin of significant proportions In the other instance, there will be periods between the two peak concentrations during which no streptomycin is demonstrable in serum. This free period may be of significance in allaying toxic reactions, but other studies to determine the validity of this variation have not been performed

Under certain other circumstances, toxicity increases, for example, following intraspinal injections and in the presence of pre-existing kidney damage

Many of the untoward side reactions are reversible, and appreciable numbers are clinically unimportant. Some reactions are irreversible and may impose a hazard upon the individual greater than the hazard of the infection being treated.

TABLE 80

Toxic manifestations of streptomycin encountered in treatment of 1,751 tuberculous patients

Regimen	1.8–2.0 gm 60–120 days*	1 0 gm 120 days	1.0 gm 120 days	0 5 gm 120 days
Number of daily injections	5	5	2	2
Number of patients treated	848	321	445	137
TOXICITY	INCIDENCE			
	per cent	*per cent*	*per cent*	*per cent*
Vertigo	76.5	34 3	23 1	5 8
Caloric stimulation, absent response	35.8	10.3	5 4	0.0
Caloric stimulation, diminished response	28.6	23.9	25 2	22.8
Hearing diminution, voice	2 2	0.3	0 2	0.0
Hearing diminution, audiometer	15 2	4.0	9 5	11.7
Renal function, reduction	9.6	7 8	5 8	2.2
Albuminuria	23 2	17 1	11.0	15 3
Dermatitis, severe	3 7	1.2	1 1	0 7
Dermatitis, mild	8 8	5.6	4 7	2 2
Eosinophilia, 6 per cent or more	59 7	36 8	34 6	31 5
Fever	4.9	0.0	1 8	0 0
Blood dyscrasia	1 0	0.9	0 7	0 7
Compelled cessation of therapy	8 5	5 0	2 0	0 0

* The percentages in this column are the corrected and combined figures from columns 1 and 2 of the original Veterans Administration table. It is considered fair to combine them, as total daily dosage and number of daily injections in the Veterans Administration columns are the same; only the duration of treatment (60 and 120 days) differs

ACUTE TOXICITY

The acute toxicity of parenterally administered streptomycin in the human has not been well described Molitor, Graessle, Kuna, Mushett, and Silber (2) have shown in animals that the LD50, with purified materials, is about 1.5 gm/kg body weight. Although no studies using single amounts of this order have been made in the human, the reported acute disasters

from the use of streptomycin (3, 4) have resulted from total daily administration of only 6 to 8 gm in divided intramuscular doses. Thus in the human, the critical toxicity seems to be in the range of 150 to 200 mg/kg; in clinical practice it is scarcely necessary to approach these amounts per day. The evidences of acute toxicity in the human are most often those of an acute reduction of kidney function. Other indications of acute toxicity have been variously described, including listlessness, semistupor, decreased respirations, slowed cardiac rate, and, rarely, coma; all of these indicating that streptomycin in large amounts depresses the medulla. Finally, at least one case of thrombocytopenia (3) has been reported with a daily dose of 6 gm. No antidote for streptomycin toxicity has been described.

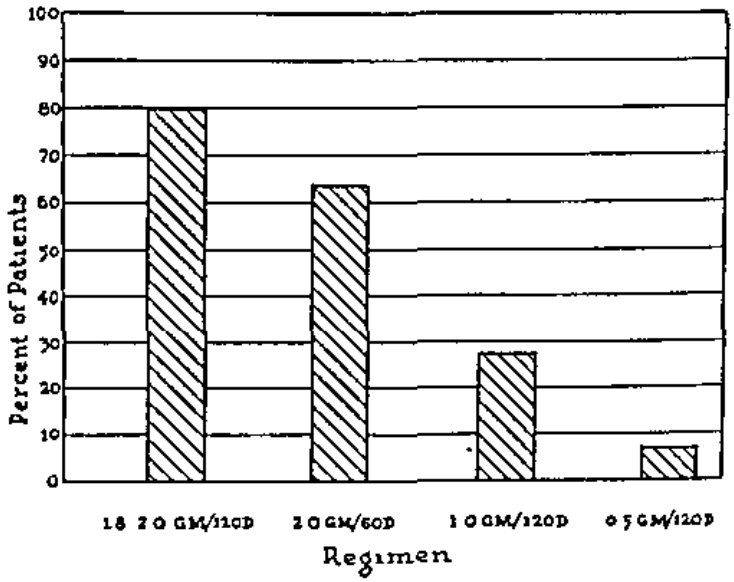

FIG 86. Effect of amounts of streptomycin and treatment period upon the percentage of toxic manifestations

CHRONIC TOXICITY

Toxic reactions in the human from prolonged exposure to streptomycin can be conveniently divided into five categories. They will be described in order of their importance to the host. It must be emphasized again that all are manifestations of chronic toxicity and that, in general, with dosage of 1 to 3 gm daily, commonly necessary in short-term therapy of acute infections, toxicity is not a problem. If a reaction does occur within a short period, it will fall into one of the categories to be described. Insofar as is possible, descriptions of reactions due to impurities formerly present in commercial preparations of streptomycin will be avoided. Adequate descriptions of these reactions, including that produced by the histamine-like substance associated with preparations of streptomycin in use prior to 1946, can be found in various papers cited in the references. An attempt will be made to distinguish patent evidences of toxicity due to impurities

not yet separated from commercial preparations from those due solely to streptomycin itself. In many of the categories this cannot be done with accuracy. The five general types of toxic reactions are: (a) a neurologic disorder of the eighth cranial nerve characterized by a loss of labyrinthine function and, rarely, by deafness; (b) a variety of irritative phenomena observed following topical and parenteral administration; (c) a nephrotoxic effect; (d) various manifestations of hypersensitization; and (e) a group of ill-defined complications of unknown importance.

Eighth cranial nerve

The principal value of streptomycin will continue to be in the therapy of tuberculosis. As it is necessary to provide the tuberculostatic agent for several weeks to attain the desired result, and as prolonged administration of streptomycin is frequently complicated by a unique derangement of the eighth cranial nerve, the neurologic reactions assume greatest importance. Together with the development of resistant organisms, this complication imposes a most serious hazard to prolonged use of the drug.

VESTIBULAR

The toxic effects of streptomycin on the human nervous system center about disturbances of function of the eighth cranial nerve. Though both vestibular and auditory functions are altered by the agent, it is the equilibratory mechanism which is more sensitive and more frequently and more permanently damaged. It is noteworthy that there is wide variation in degree of disturbances.

Symptoms of vestibular disarrangement lag far behind evocable signs. The most commonly encountered symptom is a sensation of giddiness in the upright position. This is distinguished by the absence of rotatory sensation. It is rather a misinterpretation of movement, so that there is a failure to appreciate termination of motion; the patient "mentally past points." In addition to this complaint the patient finds that sitting up, or standing, is possible only with conscious effort, and generally not at all possible with the eyes closed.

These acute vestibular symptoms are usually ushered in by a generalized headache of moderate intensity and short duration, lasting only a day or two. During the course of acute equilibratory difficulty, nausea and vomiting are commonly noted, usually precipitated by efforts at coordinated movements. Vestibular symptoms run an acute course lasting 7 to 10 days and then, often overnight, give way to a chronic state in which only sudden rapid movements elicit the linear vertigo.

With subsidence of the acute phase, there remains a state of vestibular hypofunction which arouses no symptoms in the patient confined to bed.

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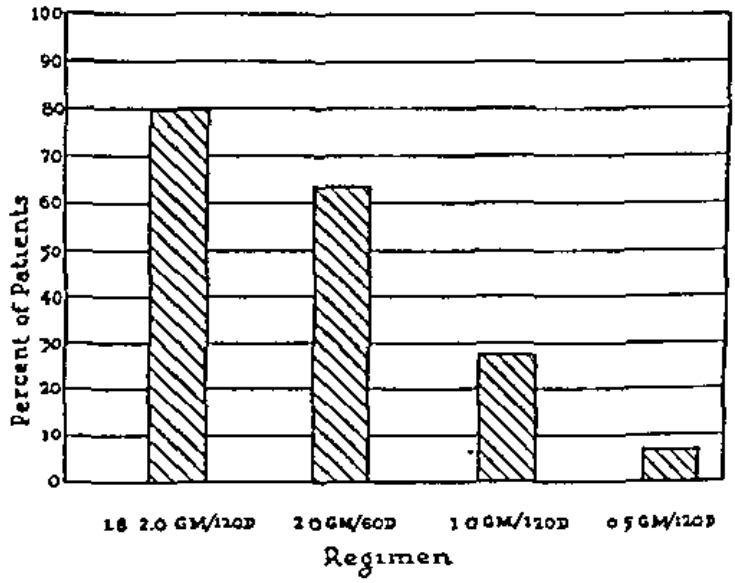

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CHRONIC TOXICITY

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characterized by vertigo and spontaneous nystagmus, the usual picture of the toxicity is depression.

There is wide variation in appearance of vestibular dysfunction in humans, even after the same dose of streptomycin. Many workers have shown that doses of a gram a day, or less, are tolerated by most patients for as long as 4 to 6 weeks without clinical toxicity. McDermott (3) found that signs of depressed function appeared after 4 weeks on 1 to 2 gm a day and late in the third week on 3 gm a day. There are, of course, many exceptions; reactions have been reported as early as the seventh day and as late as 6 months, and many individuals never demonstrate abnormal balancing mechanisms. Explanation for the failure of development in significant proportions of patients receiving large daily doses for long periods cannot be offered

The effect of total daily dosage upon the incidence of vestibular damage is impressive. It appears that, on a schedule of more than 1 gm a day, administration of 50 to 60 gm produces toxicity and the reaction is apparently a direct function of total dosage. This is shown in striking manner in table 80 and in figure 86. The series of cases include only those with presumably normal kidneys who had no other central nervous system disease and in whom streptomycin was given only intramuscularly. The incidence of subjective vertigo with 1 gm is about 29 per cent and with doses of 1.8 to 2 gm it is 76 per cent. With further reduction of daily dose to 0.5 gm daily, less than 10 per cent of the patients note vertigo. Objective evidence of disturbed balancing is also shown to be less after 0.5 to 1 gm daily than that following 1.8 to 2 0 gm, the reduction being fourfold to sixfold.

With inflammation of the brain and/or its coverings, streptomycin more readily penetrates the hematocephalic barrier Hence neurotoxic signs and symptoms appear earlier, probably because of higher local concentrations. With intrathecal injections of streptomycin, in which the same situation obtains, the eighth nerve again is damaged earlier and more frequently. It has been observed that vestibular damage has occurred in individuals as early as 4 days in this circumstance In instances of delayed excretion of streptomycin because of kidney disease, blood and subsequently central nervous system concentrations of streptomycin are also maintained at greater levels, increasing the incidence

The dissociation of nystagmus and vertigo from past pointing and rebound phenomena indicates damage to central structures, that is, the vestibular nuclei, rather than to the labyrinth or vestibular branch of the eighth cranial nerve. Pathological studies attempting to localize the damage causing vestibular hypofunction have not been fruitful Two of five patients dying after large doses of streptomycin and vestibular hypofunction showed necrosis of the inferior vestibular nuclei, according to Stevenson,

Attempts to move suddenly, to balance on a chair, or more important, to walk, bring out the latent defect. There is genuine ataxia, producing staggering, which decreases with practice, primarily in use of the eyes as vestibular organs. This second phase of vestibular disturbance lasts about 60 days on the average and then merges gradually with a state in which symptoms are latent, completely compensated but evocable by closing the eyes. Second attacks of acute symptoms in the same patient have been reported.

The first recognizable *signs* of damage to equilibration from streptomycin are evidences of "mental past pointing"—at the end of a movement, the eyes tend to drift a bit before focusing. In extreme cases there may be pendulum movements of the trunk, like those seen in a bilaterally labyrinthectomized animal. As the acute phase proceeds, the patient may not be able to sit up without support. The Romberg test is positive, without consistent direction. Spontaneous nystagmus, though reported by several observers, is unusual (4). It should be noted that although the patient has a sensation of continued motion after stopping, no true past pointing is actually noted by the examiner.

When the acute phase of vestibular toxicity subsides, signs of hypofunction gradually diminish. The ataxic gait improves, pendulum movements of the trunk and delay in focusing the eyes disappear, and in most cases the Romberg test becomes normal. In extreme cases of toxicity, however, there is permanent ataxia in the dark and a positive Romberg Decrease in muscle tone has been noted by one group (5).

Laboratory evidence of vestibular derangement depends on reduced response to labyrinthine stimulation, that is, to caloric, rotation, and electrical stimulation tests.[1] Diminished response is evidenced by slowing, delay of appearance, or absence of nystagmus after stimulation by ice water, by rotation, or by galvanic current. Electrical measurement of rate of nystagmoid eye movements in animals reveals slowing even when the movements appear after a normal interval, long before delayed nystagmus or ataxia is noted (6).

The abnormality of labyrinthine function is a bilateral symmetrical depression. Although instances have been reported of an irritative effect,

[1] Practical testing of vestibular function of a bedridden patient is best accomplished by Kobrak caloric test or some modification of it A normal labyrinth stimulated by ice water in the external auditory canal produces nystagmus in somewhat less than a 40 second latent period Nystagmus, once initiated, persists at least twice as long as the latent period. Delay in onset of nystagmus or reduction in its duration indicates depression of labyrinthine function. Other methods of recognizing abnormal vestibular function are the electrical stimulation and rotation tests These are needed only in detailed studies upon the subject, as neither offers a better diagnostic aid than caloric stimulation

sound which may persist for 14 days after the drug is discontinued The occurrence of high-pitched instead of low-pitched tinnitus has been noted; this apparently does not lead to deafness (5).

Deafness occurring in the course of therapy with streptomycin has been studied audiometrically. Brown and Hinshaw (5) emphasized the incidence of low-tone deafness, but Fowler (10) has shown that high-tone deafness also occurs, though less often. All deafness noted has been over 35 decibels. It is noteworthy that audiometrics are normal until tinnitus is experienced (4). Early recognition of hearing loss, in the presence of tinnitus, cannot be diagnosed without the aid of an audiometer because first loss is usually outside the range of conversational tone.

Measurable hearing loss is observed in 4 to 15 per cent of patients receiving streptomycin for longer than 7 days (see table 80). The variation depends almost solely upon total daily dose, but duration of exposure may also play a role in the production of the defect Generally, the incidence of deafness is higher in those patients who receive larger amounts of streptomycin. It is significantly higher in those who have kidney disease and in those receiving streptomycin by the intrathecal route. In both instances high concentrations of streptomycin within the central nervous system will be attained and maintained.

Liquefaction necrosis in the ventral cochlear nucleus of four patients dying of meningitis, who had received streptomycin, was described by Stevenson, Alvord and Correll (7). They also noted similar changes in the brains of three dogs given large doses of streptomycin. Others (2, 8), studying human and animal material, have failed to demonstrate these pathological changes.

Diminished auditory function is less important than vestibular changes, for several reasons: the incidence is less; there is a premonitory symptom—tinnitus; when the drug is discontinued at the appearance of tinnitus, no permanent impairment of hearing ensues (4, 5, 11). Practically, auditory apparatus damage may be avoided by keeping the dose of drug at the

itself and not by impurities associated with it in its production (2, 4).

Local reactions

For practical purposes streptomycin is generally administered by the intramuscular route, the usual site of inoculation being the buttock. Occasionally, following a single injection, pain, swelling, and induration result at the site of inoculation. Onset of the reaction is immediate and there is a short-lived, benign course, measurable in hours or a few days at most.

Alvord, and Correll (7). Investigations of other observers (6, 8, 9) working with post-mortem material, both animal and human, have failed to confirm this finding. Studies of the labyrinth itself and the vestibular branch of the eighth nerve are now under way.

Demonstration of the characteristics of vestibular toxicity due to streptomycin has had profound effects on the use of the drug. The deliberate administration of amounts which have been shown to cause permanent loss of at least the linear modality of vestibular function is logical only where its use is definitely lifesaving. In such a situation, vestibular damage is of little importance.

Patients who have received amounts that produce equilibratory defect eventually accommodate well enough to it to show significantly little clinical impairment. This is due entirely to the ability of these patients to use maximally extravestibular means of determining position and movement, that is, ocular and deep proprioceptive sensation. The resulting loss of balancing mechanism is a serious handicap, even with reasonably good adaptation, in patients who depend on fine coordination for their livelihood. Patients in the younger age groups (2 to 30 years) compensate for the vestibular defect readily, usually within a few months after onset of the damage. In the older age groups, compensation is not readily attained. This is due probably to a combination of factors, including inadequate vision and failing muscular coordination from prolonged underuse (hypotonicity).

The toxic effects can be avoided. Wherever possible, the dose of streptomycin should be kept at or below a gram a day, with proportionate adjustment for the patient's size and kidney function. Careful observation of the patient, preferably by means of frequent caloric tests, should be continued throughout administration of the drug, particularly when prolonged use is contemplated. Appearance of symptoms of vestibular hypofunction, such as ataxia, or of headache, should suggest neurological toxicity and lead to discontinuance whenever feasible. Fowler (9) has shown in animals and humans that antihistaminics protect measurably against vestibular damage. This deserves further study before clinical deductions can be made Protection may also be afforded by desensitization, using graduated doses of the drug This latter is, of course, not practical clinically because of the development of bacterial resistance. There is no specific treatment that will alleviate the vestibular defect once established.

AUDITORY

Streptomycin also has toxic effects on the auditory apparatus These are indicated by tinnitus, appearing 1 to 10 days after the drug is started, and followed by deafness of some degree after a few days if the drug is continued. The tinnitus is described as a continuous low-pitched, roaring

nervous system of the adult invariably gives rise to some degree of pain and produces pleocytosis in the spinal fluid. The pain is more severe and constant than can be accounted for by trauma associated with the procedure and is less acute but of the type described following injection of larger amounts. The degree of pleocytosis varies. As the material is not given intrathecally without evidences of central nervous system infection, it is difficult to evaluate the exciting cause of pleocytosis. In the few studies upon normals, it has been noted that as many as 200 to 400 cells per cubic millimeter may appear after single injections.

In infants and children similar reactions occur, but safe intraspinal dosage is less precisely defined Single injections of 2 mg/kg to a total maximum of 25 mg are reasonably free of irritative potentialities, though pain and convulsions have been observed The recommended dose in children weighing between 12 and 25 kg, regardless of central nervous system infection, is 25 mg. Adult dosage can be utilized in children weighing more than 25 kg.

Many reactions have been reported following intrathecal administration but have not received adequate study for complete understanding. Headache, vomiting, and nausea are frequently encountered with 100 mg or more streptomycin and occasionally with less. Cephalalgia, often in the cervical region, once initiated, persists for many hours and may be noted for as long as the repeated injections are continued. Somnolence has been observed; and slowing of respirations, temporary retention of urine, and nystagmus may persist for as long as 12 hours after an injection. Delirium, rise in temperature, cyanosis, dyspnea, and bradycardia have also been reported. All of these reactions are probably due to the irritant effect of streptomycin upon the brain stem.

The maximum amount of streptomycin that can be administered intrathecally in a single injection to the human is unknown. In experimental animals (monkeys and cats), streptomycin has proved to be unusually irritating in large doses, but, as has been observed clinically, irreversible reactions are set up only with the use of amounts considerably greater than those necessary for therapeutic purposes. Ataxia, inability to stand or sit, tremor of head and spontaneous nystagmus, decreased cortical activity, and convulsions have been observed after intracisternal and intracerebral injections of more than 1 gm streptomycin. Amounts of more than 200 mg would never conceivably be necessary in the human. One milligram per kilogram body weight with a maximum of 50 mg generally provides adequate concentrations of streptomycin in spinal fluid, yet is associated with few severe reactions.

The production of pain and pleocytosis cannot be avoided with intrathecal administration of streptomycin. The irritative reaction is probably

The irritation is not followed by systemic reaction, and specific therapy is not required. The agent may be continued by the simple expedient of utilizing another muscle group for the depot. The reaction is observed after the injection of any of the various salts of streptomycin, and no commercially available material is completely free from this potentiality. In general, after intramuscular administration of streptomycin, local reactions are no more frequent than that observed following administration of aqueous penicillin.

In direct contrast to the safety with which streptomycin may be administered intramuscularly, serious and often irreversible complications frequently occur at the time of, and subsequent to, intrathecal injections (table 81). The clinical picture of such reactions is variable, unpredictable, and on many occasions not separable from neurologic abnormalities

TABLE 81

Reactions after intraspinal injections of streptomycin in seventy-five patients with tuberculous meningitis

INTRATHECAL DOSE	NUMBER OF PATIENTS	TEMPORARY PARAPLEGIA		SEVERE NERVE ROOT PAIN WITH ABNORMAL REFLEXES	
		Number	Per cent	Number	Per cent
mg					
50	17	0	0 0	1	5 9
100	50	3	6 0	2	4.0
200 or more	8	3	37 5	0	0.0
Totals	75	6	8 0	3	4 0

of the disease being treated. Intraspinal injections of 100 mg or more of streptomycin in the adult are frequently followed by moderate to severe neurologic disturbances (table 81) These are marked peripheral neuropathies, hyperactive deep reflexes, and severe root pains, often controllable only with narcotics. The pain characteristically has an abrupt onset with the first few drops of the injection, is sharp and stabbing, and radiates down both legs The sensation, worse usually on the dependent side, persists for several hours and may last for 24. In a significant percentage of cases, paraplegia develops following intrathecal administration of 100 mg or more Six instances of transverse myelitis (table 81) were observed in a small series of seventy-five cases (11), and it was necessary to interrupt therapy in all because of the complication. In five of the six, reinstitution of intrathecal therapy, using lesser amounts, was accomplished uneventfully at a later date, and fortunately the paraplegia was temporary in each.

Streptomycin administered in doses smaller than 100 mg into the central

of streptomycin, cylindruria ceases promptly. The appearance of these sedimental elements throughout a treatment period occurs intermittently or is constant. Hematuria has not been observed with cylindruria. Urinary casts alone are of little significance, but in few individuals they are followed by other evidences of kidney irritation, including albuminuria and decreased renal function.

Albuminuria is observed with long-term administration of streptomycin in about 20 per cent of all cases (table 80). Although indicative of renal irritation, it, like the appearance of increased numbers of sedimental elements, has not been considered a contraindication to continued therapy. Rarely does the degree of proteinuria reach appreciable amounts; ordinarily only small amounts leak (3 to 5 gm a day or, more grossly, 1 to 2 plus, by the heat or acid test). Like the presence of increased numbers of casts, albuminuria does not excite symptoms. First evidences of proteinuria can be expected within the first week but usually come on at a later date.

The figures in table 80 indicate that some reduction in renal function occurs in a significant percentage of cases (5 to 10 per cent) and was of sufficient clinical importance in the represented series that streptomycin was discontinued. Irreversible damage, of serious import, presents itself less often (1 to 2 per cent). Evidences of interference in tubular function become apparent with decreasing values of urea clearance, phenolsulfonphthalein excretion, in retention of nitrogenous products in blood, and in the power of the kidney to concentrate fully or to dilute urine. As these findings appear relatively slowly, the trend of the pathological situation can be accurately determined prior to the decision to interrupt or to discontinue completely further administration. Because the process is insidious, only with careful laboratory observations can the damage be discovered and assayed. With cessation of streptomycin, it is usual for the function tests to return toward normal and many return to complete normality. Irreversible damage may occur with continuation, but this is not necessarily observed in all instances

The incidence of serious complication is appreciably higher in patients who have pre-existing kidney disease of any type. In a series of patients with genito-urinary tuberculosis treated at the Bronx (N. Y.) Veterans Administration Hospital (12), the incidence of severe renal damage was twice that observed in similar studies on patients with pulmonary disease. It is conceivable that in this circumstance, retention of streptomycin in blood may add to the irritant effect upon the damaged kidney. This situation is ill advised, not only because further kidney damage is thus potentially excited, but also because the relationship between high blood levels of streptomycin and early occurrence of grave eighth cranial nerve damage is manifest. On the other hand, the existence of known kidney disease does

due to the chemical, and it is unlikely that completely purified preparations will be entirely innocuous to the central nervous system coverings. The reactions can be avoided by the simple expedient of not using the intraspinal route for administration. Severe reactions can be prevented by injecting single amounts of less than 100 mg and as dilute as is practicable. With proper indication for the intraspinal use of streptomycin, the irritative phenomena must be endured.

Streptomycin can be safely introduced into other body cavities without harmful effect Concentrations of the material of 10 to 25 mg/ml saline is nonirritating to either the pleural or peritoneal serosa As much as 0.1 gm/ml can be deposited in the peritoneal cavity without hazard, and 0 5 gm/ml in urinary bladder yields no ill effects. Topical application to wounds, utilizing 0.2 to 0 5 mg/ml saline does not produce undesirable side effects and ten to twenty times that concentration can be nebulized safely Maximal concentrations have not been described. Solutions containing as much as 20 mg/ml can be used locally in the eye without significant danger, and similar concentrations applied to the external auditory canal and in the nose are nonirritating.

Pain and irritation at the site of subcutaneous inoculation limit the usefulness of this route. As absorption from a subcutaneous depot is little different from that of an intramuscular one, this method of administration is rarely utilized. Complications do not occur from the ingestion of streptomycin. Instillations into joint spaces and into the pericardium have not been reported, but in view of the experiences recited above, it can be presumed that streptomycin in similar concentrations would provoke no serious irritative phenomena

Kidney reactions

Interference with renal function produced by streptomycin constitutes an important disability, although serious reduction in kidney function is not common Cylindruria may frequently appear within 48 hours after streptomycin is first administered parenterally (table 80) Hyaline and granular casts are found in varying numbers, and if therapy is continued for longer than 7 days, most individuals (50 to 70 per cent) will excrete them. The number found in a centrifuged urine specimen varies from 3 to 20 per high-powered microscopic field In acid urine (pH 5), the number of casts increases. With alkalinized or neutral urine, the cylindruria is minimal or absent. It is unlikely that the maintenance of alkaline urine assures protection from kidney damage due to streptomycin. Casts are more soluble in such a medium It is virtually certain that numerical reduction in a medium of pH 7.5, or higher, merely indicates their dissolution in the urinary bladder, after their formation in the kidney tubules. With cessation

dosage of streptomycin can be reduced, interrupted, or discontinued entirely.

Manifestations of hypersensitization

Streptomycin, like other chemotherapeutic agents, is capable of being antigenic. Various evidences of sensitization phenomena are commonly observed in patients receiving it for 5 or more days. The reactions include eosinophilia, fever, rashes, and, on occasions (1 to 2 per cent), exfoliative dermatitis (table 80). Of these, eosinophilia alone is the most common. Though each may appear alone; all may occur together. The production of allergic reactions is a property of streptomycin itself, as Molitor *et al.* (2) and Farrington (4) have observed in their studies with "pure" materials. The development of allergy to streptomycin in humans apparently is not dependent on hereditary factors.

Eosinophilia (table 80) of more than 5 per cent is seen in more than 30 per cent of all individuals receiving streptomycin. The numbers of these cells vary throughout the exposure period. They may appear early and remain in elevated numbers; they may appear intermittently, reaching, on occasions, as many as 70 per cent of all circulating white blood cells; or they may be present for only a few days, their occurrence coming on at any time during the administration of streptomycin. Usually the eosinophilia begins between the 9th and the 15th day of exposure, but its onset within 2 days and after 90 has been reported. Following discontinuation of streptomycin, the number of eosinophiles decreases progressively to normal limits. In itself, eosinophilia is not a reaction that requires cessation of streptomycin, nor does it necessarily indicate serious sensitization Its presence is disturbing only because the phenomenon represents an allergic state, and more serious untoward allergic reactions are potential in the group exhibiting it

The incidence of eosinophilia is less in individuals receiving 0 5 to 1 0 gm streptomycin daily for 120 days than in those receiving more than 1 gm (table 80). This differential, due to the size of total daily dose, is not an unusual allergic phenomenon There is, thus, a distinct advantage in using small amounts of streptomycin, for with a lessened incidence of eosinophilia, potential danger from other more serious allergic reactions is reduced Duration of treatment also affects the incidence of reactions—the longer the exposure, the greater the incidence. This observation probably reflects the influence of total drug administered, not extent of exposure.

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hemorrhagic. Many are nonpruritic. In general, the eruptions are similar to the dermatitis seen with other drug eruptions and, quite likely, some fit into the group of 9th day erythemas of Milian. In these, at the height of the rash, there is a fall in blood pressure, associated with constitutional symptoms such as fever, arthralgias, and headache. These latter symptoms subside within a few hours after cessation of streptomycin but may persist for 3 to 4 days. Among the group in whom there are no systemic complaints, the rash persists for 1 to 10 days. A mild febrile response is observed in somewhat fewer than half the cases with eruption.

Like other drug eruptions, the exanthema begins as a localized area on the extremities or head but usually spreads to involve a major portion of the body. After spread, superficial scaling follows on occasions. Antihistaminic agents control the pruritus when present. Streptomycin usually can safely be continued during the episode of eruption and, despite continuance of the drug, the rash gradually subsides. When constitutional symptoms are unduly severe and when the rash progresses in severity and in symptoms, cessation of streptomycin is indicated. Its administration must be immediately discontinued when exfoliative dermatitis supervenes. Streptomycin can often be resumed in individuals whose therapy is interrupted by a severe toxic eruption. Single small test doses (a dose as small as 10 mg may excite a severe recurrence; 10 μg is recommended) after 3 or 4 weeks will indicate the status of the patient's response to the antigenic properties of the material. When the test dose fails to elicit a rash, streptomycin can be reinstituted, but with caution

Drug fever is uncommon, its occurrence being noted in less than 5 per cent of cases. The great majority of such cases are associated with a toxic eruption. The fever appears with, and its course parallels that of, the rash, common subsidence usually being observed despite continued administration of streptomycin. The temperature rarely reaches 103°, and it can be controlled with salicylates. It is of interest solely because it is another manifestation of the sensitivity in an individual; its presence with an eruption is of no prognostic significance.

High sustained fever (102 to 104°), coming on after 5 to 7 days, has not been reported following administration of highly purified streptomycin. With the less purified materials, this more typical "drug fever" may occur and persist until streptomycin is discontinued.

Dermatitis venenata has been reported many times among individuals habitually handling streptomycin. It is seen particularly in nurses who prepare solutions for injection The dermatitis comes on after protracted contact and appears usually on hands and lower arms, rarely involving face and eyes. By use of gloves, the immediate problem in the medical personnel can be avoided. In those who have developed the dermatitis,

"desensitization" occasionally can be carried out to complete relief of the pathology, but more often is unsuccessful. The disease, of course, is a self-limited one, though uncomfortable during its existence, provided the susceptible individual remains out of contact with streptomycin.

Speculation as to mechanisms that produce evidences of hypersensitivity is of interest. It is known that streptomycin has the property of combining with plasma protein to a varying, but usually slight, degree. As antigens in general presumably must be partly protein, it is conceivable that a streptomycin-albumin complex is formed in plasma, and that the complex becomes antigenic, with streptomycin acting as a haptene. Although specific antibodies, induced by other protein-bound chemotherapeutic agents, have been described, none has been discovered in response to therapy with streptomycin in the human. It is unknown also that the uncombined chemical in serum has an antigenic property, but other non-protein antigens are rare indeed. Be that as it may, allergic reactions due to streptomycin follow patterns observed with other sensitizing protein agents, and therefore methods to avoid, prevent, or treat any single phenomenon should be patterned after general principles described for the other agents.

The hypersensitivity phenomena induced by streptomycin are commonly first observed 4 to 5 days after the initial dose, and it is probable that the individual becomes sensitized immediately after the first dose. When the initial parenteral therapy is continuous, acute severe reactions are distinctly uncommon. In fact, reactions will generally subside despite continuation, probably by the direct process of "desensitization." Consequently, in the presence of a mild or moderately severe allergic reaction, streptomycin may be continued without fear of disaster, the reaction being self-limited. On occasions, exfoliative dermatitis appears during first exposure to streptomycin In the presence of this explosive cellular antibody-antigen reaction, streptomycin must be discontinued, for further therapy is dangerous. If a reaction of any degree occurs during the first course of streptomycin, there is greater likelihood that a second exposure will excite more frequent and more severe allergies

Despite the immediate safety of continuing streptomycin in the presence of evidences of sensitization, the work of Rich *et al.* (13) indicates that this situation may be a precursor of generalized vascular disease. In the presence of evidences of the sensitized state, consequently, the physician must weigh well the possibilities. If the infection being treated threatens life, the decision to continue streptomycin is easy. On the other hand, if the disease is not associated with high mortality, to continue streptomycin may impose a great disadvantage upon the patient—a disadvantage not immediately obvious but potentially serious.

Miscellaneous reactions

A multitude of other untoward side reactions have been recorded following administration of streptomycin. The majority of these are clinical observations, frequently made, to be sure, but so incompletely studied that explanations for the phenomena are either illogical or impossible.

Observed particularly in tuberculous patients receiving streptomycin for long periods is a rather remarkable degree of euphoria. The sense of well-being is real, usually marked, and intimately associated with the administration of the agent. It cannot always be associated with improvement in the tuberculous process, for it appears and continues in some whose disease is not grossly affected by streptomycin. Further, it often begins prior to any change in symptomatology. *There is no explanation from this change* in personality, but it is unlikely that it results from suggestion alone.

The appearance of mild conjunctivitis during administration of streptomycin has been reported. The inflammation is benign, short-lived, and not associated with pus formation. Excessive tearing, and, on occasions, photophobia, accompanies the conjunctival injection. Although it is likely that the phenomenon is an allergic manifestation, many instances of conjunctivitis occur without eosinophilia, fever, or rash It may appear at any time during a course of treatment. As with euphoria, the complication is unimportant, and therapy need never be discontinued because of it. The reaction is not the same as the conjunctivitis reported with dermatitis venenata.

Paresthesia of lips and fingers are also subjective observations frequently made by patients receiving streptomycin for prolonged periods The numbness and tingling is restricted to the area of the orbicularis oris muscle and to the tips of the fingers It persists for many days or weeks, and although it does not obviously alter either taste or appetite, it may interfere with *enjoyment of meals. Pain is never a part of the sensation* The paresthesia always disappears when streptomycin is discontinued, and it may subside during continuance of the drug No importance can be attached to this rather common complaint Neither is there an explanation for its existence No serious sequelae have been reported as a result of it

Headache, nausea and vomiting, and gastro-intestinal discomfort have been reported (1, 14). The relative incidence of the last is directly related to daily dose (fig 87) Again there is no obvious explanation for occurrence of these reactions Therapy, on occasion, has to be discontinued because of the severity of the nausea and vomiting, both symptoms subside after cessation of therapy. In some patients, however, nausea and vomiting fail to reappear with reinstitution of therapy Because no study of the problem has been recorded, further description is useless It is pertinent to add that nausea, vomiting, and headache are symptoms often associated

with delayed anaphylactic phenomena, and it is possible that, among individuals receiving streptomycin, these reactions occur in the group exhibiting other but subdued evidences of allergy. The importance of the coincidence has not been determined.

Streptomycin may occasionally act as a bone-marrow depressant, with the production of blood dyscrasias. Although pancytopenia has developed during therapy in a sufficient number of cases to warrant assumption that its action is directly upon bone marrow, there has been no report of a case whose reaction has been well followed with bone marrow studies for final proof. Most instances of anemia and granulocytopenia have been in individuals with disseminated tuberculosis or with an acute infection that may well have been associated with bacteremia and possible septic emboli-

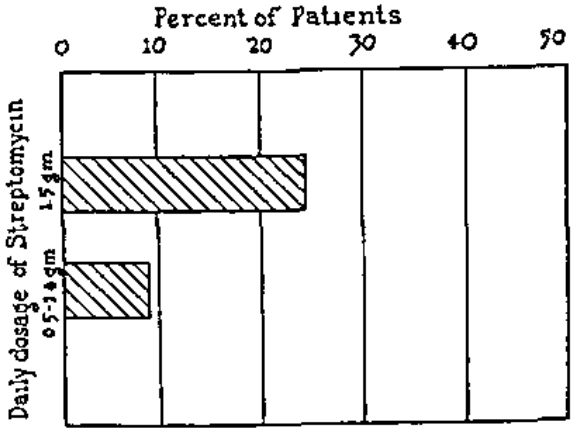

FIG. 87. Relative effect of daily dosage of streptomycin upon the percentage of toxic manifestations.

zation in bone marrow. Bunn (11) referred to a case of miliary tuberculosis in whom agranulocytosis developed during therapy with streptomycin and a return of white blood cells occurred after cessation of streptomycin despite progressive and fatal disease. McDermott (3) referred to a patient of Adcock's who developed thrombocytopenia while receiving 6 gm of streptomycin daily. Recovery was complete following discontinuance of the therapy. From the reports of the streptomycin committees of the Veterans Administration, Army, and Navy (1), blood dyscrasias occur with an incidence of 0.7 per cent, but the completeness or type of bone-marrow depression is not described. These and many other reports indicate that streptomycin indeed may affect the bone marrow, but final decision of its seriousness awaits further study.

Leucopenia, on the other hand, is commonly seen during prolonged administration of streptomycin. Although there is true depression of granulocytes, the number uncommonly falls below 1,500/ml. The leucopenia is reversed during continuation of therapy. During acute episodes of sensitization phenomena, there may be, of course, leucopenia with rela-

tive increase in eosinophiles. This phenomenon is apparently separate from that of a possible true bone-marrow depressing action of streptomycin

If streptomycin can produce pancytopenia, the reaction, must be rare, there is no specific way to avoid it, and there is nothing with which to treat it once established, except to discontinue all further administration of streptomycin.

Hepatic damage has not been reported.

In Conclusion

Most patients tolerate streptomycin well, but the toxic reactions are more frequent and considerably more hazardous than those following penicillin. The allergic manifestations may conceivably predispose to disseminated vascular disease. Exfoliative dermatitis may on occasion be fatal. The nephrotoxic effect may impose permanent impairment of kidney function. The unique eighth nerve complication may be a serious, permanent, economic, and social defect in the future of patients receiving streptomycin for long periods. The incidence and severity of toxic reactions increase in direct relation to size of dose, the height of its concentration in the patient's extracellular fluids, and the length of its administration. All of these require that the physician wisely and carefully weigh the precise value of therapy with streptomycin, in any infection, against the hazards derived from use of the drug. As it is quite likely that streptomycin will be used, for the most part, in the therapy of subacute or chronic infections, such as tuberculosis, brucellosis, and subacute bacterial endocarditis, and must, in consequence, be administered for long periods, measurable in weeks, the decision is all the more difficult. For the therapy of infections such as tularemia, in which streptomycin need be given for only a few days, the *problem is simple; in small doses for short periods, streptomycin* is a safe agent. It is unlikely that the common use of pure or crystalline streptomycin will obviate the toxicity as recorded. At present there is no method of safely preventing or circumventing any one of the unfortunate side effects. Despite the complications associated with its prolonged usage in any individual, streptomycin is an established and effective antimicrobial agent. That it requires wisdom to administer detracts nothing from its value; rather it stimulates the physician to understand and evaluate more carefully the patient's disease—an advantage to both the physician and the patient.

Dihydrostreptomycin

In the constant search for a tuberculostatic material that would combine the effectiveness of streptomycin with lessened toxicity, a clinical study of the dihydro form of streptomycin was undertaken in 1947–48 (14, 15, 16).

At this writing, dihydrostreptomycin has not been adequately studied toxicologically to determine accurately its toxicity in the human following prolonged administration, though preliminary clinical trials indicate that it has certain advantages over the parent streptomycin.

The principal toxic reaction from streptomycin is the neurotoxic effect upon the eighth cranial nerve. Dihydrostreptomycin is capable of producing the same reaction in the human, but it does so after some delay when compared with streptomycin. Between 2 and 3 gm of streptomycin a day, administered parenterally, will produce labyrinthine damage within 3 to 4 weeks in the vast majority of instances. After 6 weeks, 2 to 3 gm of dihydrostreptomycin daily will evoke the reaction in only a few cases Dihydrostreptomycin seems to be distinctly less neurotoxic, in that the reaction will occur later after the use of large doses. As is true with the parent substance, eighth nerve damage occurs earlier with high serum concentrations of dihydrostreptomycin. Once initiated by dihydrostreptomycin, eighth nerve damage is less severe than that usually observed after streptomycin. In general, though, experience has not yet taught how complete, how serious, or how permanent the damage will be, nor is it possible to estimate precisely at what time the effect can be expected in any individual. Dihydrostreptomycin will also produce auditory complications; but relative safety, when compared with streptomycin, is not yet known. Like the vestibular, cochlear toxicity may be delayed and may be less severe (14).

Administration of dihydrostreptomycin is followed by appreciably fewer hypersensitivity phenomena than those following administration of streptomycin. Eosinophilia is uncommonly seen, rashes and more severe forms of allergy, such as, fever and exfoliative dermatitis, are not reported. Again the explanation for the fewness of reactions is lacking, nor is it precisely known how frequently reactions will occur. It is probable, however, that allergic reaction will occur with continued use of dihydrostreptomycin and that, as with the experience gained from other chemotherapeutic agents, the incidence will be at least 5 per cent.

An advantage of dihydrostreptomycin has been recorded. In instances of severe allergic reactions to streptomycin, and in the presence of adequate indications for its continued antimicrobial action, dihydrostreptomycin may be safely substituted for streptomycin without fear of continued hypersensitization (14, 15). Streptomycin acts as an antigen, but its antigenic property is distinct from that of dihydrostreptomycin, and there are apparently no cross-immune reactions. Consequently, in patients unable to tolerate streptomycin because of hypersensitivity, therapy may be carried on with dihydrostreptomycin.

It has long been suspected that eosinophilia, other allergic manifestations,

and vestibular dysfunction are intimately related, but no common explanation has been offered. With apparent decrease in instances of both allergic manifestations and eighth nerve damage from dihydrostreptomycin, there is strengthened evidence that the neurotoxicity of streptomycin is indeed an evidence of sensitization. It is of interest that the two materials have the same pharmacologic and antibacterial properties in humans, yet have different allergic potentials. There is but one described difference in their biologic activities that might bear a relation to sensitization phenomena—streptomycin is inactivated by cysteine, and dihydrostreptomycin is not.

Dihydrostreptomycin is nephrotoxic. The time of onset, the incidence, the severity, and the importance of pre-existing kidney disease as a predisposing factor in the production of renal reactions are not known. Similarly, the natural course of the kidney reaction, once established, cannot be described. It is not yet recognized how often or at what period cylindruria and/or albuminuria will be observed. Neither is it known how much dihydrostreptomycin will initiate the irritation. McDermott reported the death of an individual receiving 5 gm of the material daily for 54 days (14). Pre-existing kidney damage was present in this patient, and during the course of therapy she developed retention of nitrogenous products, became deaf, and died in a state of uremia. It appears, consequently, that dihydrostreptomycin contains a kidney irritant, and this fact supplies ample evidence that any individual receiving streptomycin or dihydrostreptomycin for long periods should be subjected to adequate laboratory observations, including tests of kidney function.

Dihydrostreptomycin produces more local reactions than streptomycin. It is more irritating at the site of intramuscular injection, and intrathecal administration is followed by severe reactions. Hinshaw reported partial paraplegia, urinary and fecal incontinence, and "saddle" cutaneous anesthesia in one patient receiving 50 mg dihydrostreptomycin intrathecally (15). A reaction of this severity has not been observed in an adult receiving this amount of the usual purified preparations of streptomycin. Studies of irritating properties of dihydrostreptomycin after injection into other body cavities have not been reported. Whether the irritant property can be removed with subsequent purification cannot be stated, but that great effort will be expended to remove it cannot be doubted.

As dihydrostreptomycin has almost identical pharmacologic and antibacterial properties with streptomycin (15), it is difficult to conceive of its potential toxicity being as benign as it now appears to be. Good definition of its chronic toxicity must await further studies. Until these are carefully performed and recorded, it would appear to be the better part of wisdom to administer dihydrostreptomycin with the same caution as that exercised with streptomycin. A limiting factor to the use of any type of strepto-

mycin for long periods will be the rapidity and completeness of the emergence of organisms resistant to its antibacterial effects. As organisms develop overwhelming resistance to dihydrostreptomycin as rapidly and to the same degree as to streptomycin, it may well be that the differences in toxic manifestations, possibly less frequent, certainly delayed, and probably less severe with dihydrostreptomycin, will permit somewhat greater latitude in the administration of dihydrostreptomycin. Because of the apparent lessened toxicity of dihydrostreptomycin, the importance of the emergence of resistant organisms increases, while the untoward side reactions depreciate.

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and vestibular dysfunction are intimately related, but no common explanation has been offered. With apparent decrease in instances of both allergic manifestations and eighth nerve damage from dihydrostreptomycin, there is strengthened evidence that the neurotoxicity of streptomycin is indeed an evidence of sensitization. It is of interest that the two materials have the same pharmacologic and antibacterial properties in humans, yet have *different allergic potentials. There is but one described difference in their* biologic activities that might bear a relation to sensitization phenomena—streptomycin is inactivated by cysteine, and dihydrostreptomycin is not.

Dihydrostreptomycin is nephrotoxic. The time of onset, the incidence, the severity, and the importance of pre-existing kidney disease as a predisposing factor in the production of renal reactions are not known. Similarly, the natural course of the kidney reaction, once established, cannot be described. It is not yet recognized how often or at what period cylindruria and/or albuminuria will be observed. Neither is it known how much dihydrostreptomycin will initiate the irritation. McDermott reported the death of an individual receiving 5 gm of the material daily for 54 days (14). Pre-existing kidney damage was present in this patient, and during the course of therapy she developed retention of nitrogenous products, became deaf, and died in a state of uremia. It appears, consequently, that dihydrostreptomycin contains a kidney irritant, and this fact supplies ample evidence that any individual receiving streptomycin or dihydrostreptomycin for long periods should be subjected to adequate laboratory observations, including tests of kidney function.

Dihydrostreptomycin produces more local reactions than streptomycin. It is more irritating at the site of intramuscular injection, and intrathecal administration is followed by severe reactions. Hinshaw reported partial paraplegia, urinary and fecal incontinence, and "saddle" cutaneous anesthesia in one patient receiving 50 mg dihydrostreptomycin intrathecally (15). A reaction of this severity has not been observed in an adult receiving this amount of the usual purified preparations of streptomycin. Studies of irritating properties of dihydrostreptomycin after injection into other body cavities have not been reported Whether the irritant property can be removed with subsequent purification cannot be stated, but that great effort will be expended to remove it cannot be doubted.

As dihydrostreptomycin has almost identical pharmacologic and antibacterial properties with streptomycin (15), it is difficult to conceive of its potential toxicity being as benign as it now appears to be. Good definition of its chronic toxicity must await further studies. Until these are carefully performed and recorded, it would appear to be the better part of wisdom to administer dihydrostreptomycin with the same caution as that exercised with streptomycin A limiting factor to the use of any type of strepto-

mycin for long periods will be the rapidity and completeness of the emergence of organisms resistant to its antibacterial effects. As organisms develop overwhelming resistance to dihydrostreptomycin as rapidly and to the same degree as to streptomycin, it may well be that the differences in toxic manifestations, possibly less frequent, certainly delayed, and probably less severe with dihydrostreptomycin, will permit somewhat greater latitude in the administration of dihydrostreptomycin. Because of the apparent lessened toxicity of dihydrostreptomycin, the importance of the emergence of resistant organisms increases, while the untoward side reactions depreciate.

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CHAPTER 41

ALTERATIONS IN NORMAL BACTERIAL FLORA OF MAN AND ANIMALS AND SECONDARY INFECTIONS DURING STREPTOMYCIN THERAPY

The use of antibiotic agents with highly specific, selective activity has solved many of the difficulties encountered in the treatment of infectious diseases. Many new problems, however, have been created. Some of these arise because the lives of patients are prolonged sufficiently to allow time for development of complications of the original disease; others present themselves as the result of toxic and allergic reactions, introduction of new organisms during therapeutic manipulations, changes in bacterial flora with resultant tissue invasion, and improper bacteriologic diagnosis. It is with one of these problems, the appearance of new infections during the course of treatment with streptomycin, that this chapter concerns itself.

In spite of the fact that both penicillin and streptomycin are highly antibacterial, their activity is more or less specific. Thus, penicillin is active mainly against gram-positive bacteria, whereas streptomycin exerts its greatest effects against members of the gram-negative group. Although organisms susceptible to each of these agents show varying degrees of resistance, most of the susceptible bacteria usually can be eliminated if an adequate dose of either of the antibiotic agents is given. During therapy with streptomycin or penicillin, alterations in the bacterial flora in various parts of the body may occur in addition to an effect on the specific organism responsible for an infection. Thus, gram-positive bacteria may be eliminated from the nasopharyngeal flora when penicillin is administered, and gram-negative ones when streptomycin is given.

Lipman, Coss, and Boots (5), in a study of the throat and intestinal flora of cases of rheumatoid arthritis to whom penicillin was administered daily over a period of months, demonstrated a rapid and striking change. The throat cultures of all of the patients, prior to antibiotic therapy, revealed a predominance of gram-positive diplococci sensitive to penicillin. During the course of treatment, gram-negative organisms, mainly *E. coli*, were found to be predominant. Less striking though definite changes in the intestinal flora also took place

Influence on Bacterial Flora

The influence of streptomycin on the bacterial flora of man and animals has not been investigated very extensively. The available data are limited primarily to observations on changes in the microbial population of the intestine and are reviewed below.

In mice and rats

Studies by Smith and Robinson (9) on the effect of feeding varying quantities of streptomycin to mice revealed that daily administration of 30,000 units of the drug per kilogram of body weight resulted in a decrease, within the first 24 hours, of the number of coliform organisms from a normal of approximately 100,000 to 100 bacteria per 3 mg of feces. Continued ingestion of streptomycin for as long as 3 weeks resulted in maintenance of the low level of bacterial numbers with very little daily fluctuation. When the quantity of antibiotic agent was increased to 300,000 units per kilogram (one-twentieth the maximum tolerated dose) all of the coliform group disappeared within 24 hours and were undetectable throughout the experimental period (3 weeks). Cessation of treatment produced a 1,000–1,500-fold increase in the coliform count within 48 hours. The animals that had received the small dose of drug reattained a normal number of bacteria in the feces, but those which had been given the larger quantities had subnormal counts for at least an additional 6 days. In the animals given 300,000 units of streptomycin per kilogram not only *E. coli* but all of the gram-negative organisms were eliminated, leaving only a small number of gram-positive ones such as *B. mesentericus*, *B. megatherium*, and *B. subtilis*. A similar change was observed in those receiving the smaller dose of antibiotic agent but the gram-negative flora was not eliminated so completely. In the feces of the mice fed 300,000 units of streptomycin, *B. closteroides* appeared about 96 hours after treatment began and disappeared in about 1 week. This organism was found to be thirty times more resistant to streptomycin than *E. coli* "W." Although no cultural studies were carried out, smears of the feces gave the impression that there was a reduction in the numbers of anaerobes. No evidence was obtained that any of the fecal bacteria became streptomycin-resistant during the period of treatment.

That changes in bacterial population of the intestinal tract resulting from the feeding of an antibiotic substance may not be without harm to animals has been demonstrated by Emerson and Smith (2). Administration of 160,000 to 375,000 units of streptomycin per kilogram daily in conjunction with a purified diet produced no significant nutritional disturbances in rats. When the quantity of drug was increased to between 580,000 and 875,000 units per kilogram, however, manifestations resembling those

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produce hydrogen sulfide, and loss of virulence for guinea pigs. The stools of children receiving streptomycin were found by Dalton to be free of *E. coli*. In a few instances, streptomycin-resistant strains of *A. aerogenes* were cultivated from the feces of those receiving the drug, but no other gram-negative bacteria could be grown.

It is possible that such alterations in the distribution of organisms in sites of the body where a mixed flora is usually present may occur in many patients who receive therapy with a specific antibiotic agent. In most cases, such changes in the bacterial population with suppression of certain groups of bacteria and increase in the number of others probably leads to no untoward result, and the patient's recovery is not affected. In other cases, however, these alterations in the flora result in the rapid growth of virulent bacteria, which may have been present originally only in very small numbers, and which were not susceptible to the antibiotic agent administered. If the general resistance to infection is depressed, new infections may occur following such a change. In the cases described below, use of streptomycin resulted in the elimination of one group of bacteria and allowed another, which was probably present in small numbers in certain sites, to become numerically predominant and to invade the tissues and produce new disease.

Development of New Infections

New infections arising during the course of antibiotic treatment may have a different pathogenesis than the one just described. Organisms that are not susceptible to the specific agent being used may be introduced accidentally (from the skin or from contaminated apparatus) during administration of the antibiotic substance into the pleural, peritoneal, synovial, or lumbar spaces or into the muscles or blood stream in the course of venipuncture and intramuscular injections, and may produce new infections at a time when the original disease is responding well to therapy. Infection with gram-positive bacteria during the course of streptomycin treatment, such as gluteal abscess due to *S. aureus* and empyema due to the same or other streptomycin-insensitive bacteria, have been observed in a number of clinics. This type of new disease is not the result of a change in the normal bacterial flora of tissues, however, but is produced by accidental introduction of new organisms during the manipulations incident to the proper administration of the antibiotic agent

Clinical histories

Below are presented three instances of new infections occurring during the course of streptomycin treatment and which are thought to have resulted from changes in bacterial flora induced by this drug.

present in biotin deficiency developed. The feeding of streptomycin led to a marked diminution in the numbers of coliform bacteria as well as in the total intestinal flora. This early reduction in bacterial population was followed by a rise to normal in 7 to 12 days, which was due to the development of streptomycin-fastness within the first 24 hours of the initiation of therapy.

In man

Interest in the changes produced by streptomycin in the bacterial flora of man has been centered mainly on the intestinal tract because of the usefulness of the antibiotic agent in the treatment of some primary bowel infections and for preparation of the intestine for surgical procedures. Only a few observations on this important subject have been recorded; however, and study of other areas of the body such as the nasopharynx have been neglected, on the whole. The first data on the effect of streptomycin on the bacterial flora of man are those recorded by Reiman, Price, and Elias (7), who administered streptomycin orally in varying doses and produced a marked reduction in the numbers of *E. coli* in the feces, in some patients there was a concomitant decrease in the quantity of other aerobic bacteria but no change in the anaerobic population. Cessation of treatment was followed by rapid return of the fecal flora. There was great variability in the resistance of different strains of *E. coli* to streptomycin.

Pulaski and Amspacher (6) treated a number of human beings with various types of infections of the intestinal tract with 2 to 4 gm of streptomycin by mouth and noted a reduction in the number of coliform organisms in the stool within 24 to 48 hours after therapy was started The oral administration of as little as 1 gm of streptomycin was found by Kane and Foley (3) to eliminate *E coli* from the stools of five humans within 2 days This organism was absent from the feces as long as administration of the drug was continued but reappeared promptly after treatment was discontinued. In patients who were subsequently operated upon, cultures of the colonic mucosa revealed an absence of *E. coli*. In an individual with a colostomy, lavage with streptomycin per rectum rendered the bowel segment free of *E. coli* No changes were detectable in the numbers of anaerobic bacteria or streptococci in the stools

Schwarzkopf (8) studied the effect of streptomycin administered intramuscularly on the stool flora of a typhoid carrier and noted that *E coli* disappeared from the feces in 3 days, after administration of 9 9 gm of the drug S. *typhosa* was not eliminated from the stool by as much as 23 gm of the antibiotic agent, except for a period of 24 hours The typhoid bacillus was observed, however, to undergo dissociative changes with the development of first rough and then abnormal colonial morphology, inability to

erate degree of improvement. On the day before admission, the patient began to have generalized convulsive seizures, which rapidly increased in frequency and duration until they were present almost constantly at the time of entry to an outlying hospital, where a lumbar puncture revealed cloudy spinal fluid containing gram-negative pleomorphic bacteria. Because of this finding, the child was referred to this hospital for treatment.

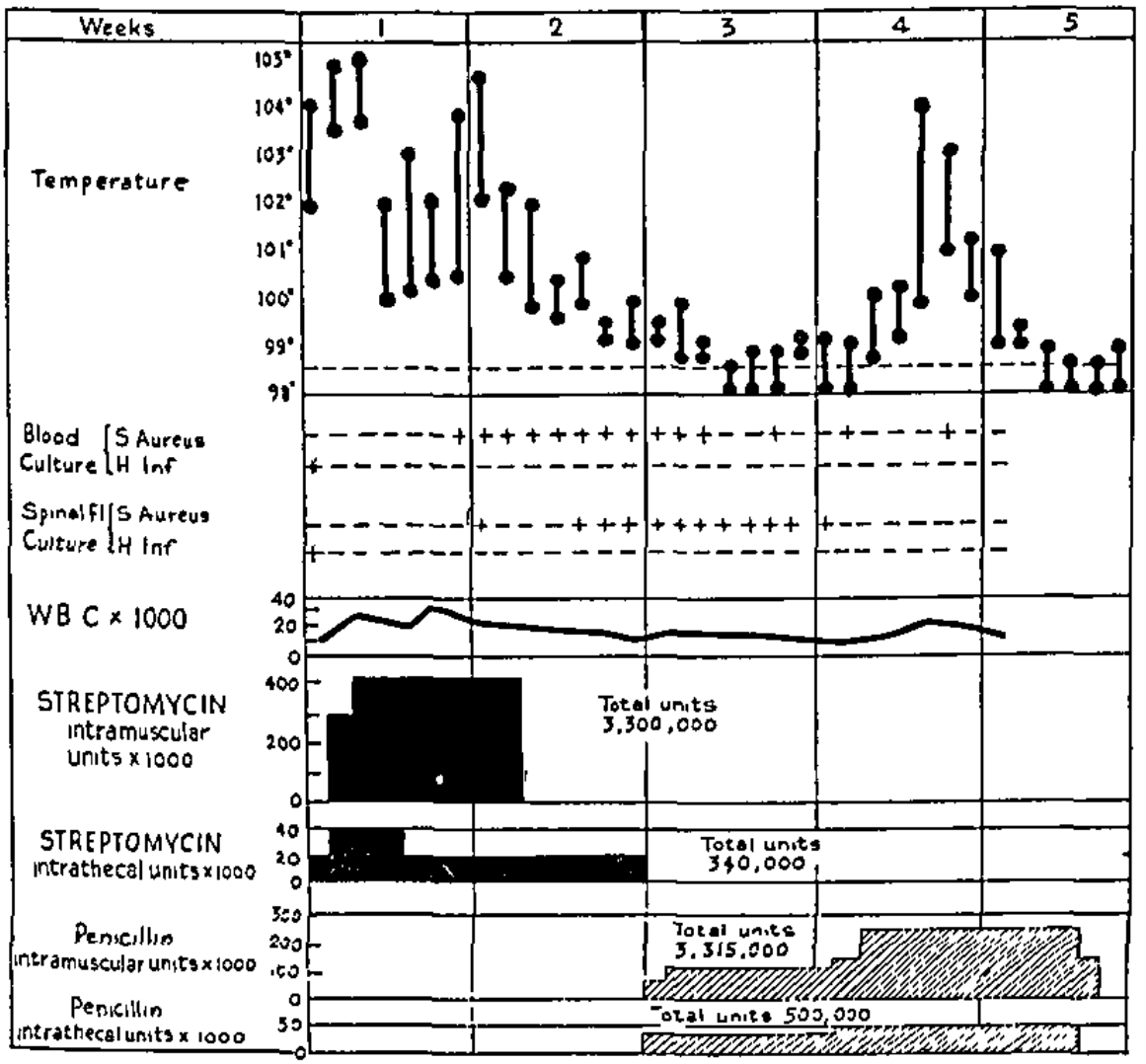

FIG. 88. A complicating *S. aureus* bacteremia and meningitis following recovery from *H. influenzae* (type B) meningitis and bacteremia (4).

On admission the patient was a well-developed, moderately obese infant, who held himself rigid, with all the extremities in flexion and the head in extension. The temperature was 102.2°F, the respirations 32, and the pulse 130. The anterior fontanelle was wide open and bulging. The lower part of the right tympanic membrane was red, and the light reflex and visibility of the bony landmarks were diminished. The eyes followed a bright light to the right but not to the left. The nose contained a small amount of

Case 1. A 3-year-old girl was admitted to the hospital because of fever and abdominal pain. Two and a half years prior to admission she had had a "kidney infection," from which she recovered in 23 days. Seven weeks before coming to the hospital, she had uncomplicated pertussis. Six hours before admission, there was a sudden onset of abdominal pain and vomiting The temperature was elevated to 101.2°, and the patient was brought to the hospital.

On admission, the temperature was 99°F, the pulse 160, and the respirations 26. Physical examination was entirely within normal limits except for slight distention of the abdomen and generalized abdominal tenderness, somewhat more marked on the right. Peristaltic sounds were diminished. No tenderness could be detected in the costovertebral angles. The white cell count was 24,000, with 84 per cent neutrophiles and 16 per cent lymphocytes. Examination of the urine revealed a specific gravity of 1.020, 2 plus albumin, and 50 to 60 white cells per high-powered field, in a catheterized specimen. The hemoglobin was 11 gm/100 ml. Culture of the urine revealed *H. influenzae*, which was neither type A nor type B.

Streptomycin, 125 mg every 3 hours, was administered intramuscularly for 5 days. The temperature remained normal during the entire course of treatment, .
the antibiot .
on the 5th d .
minuria disappeared. The white cell count fell to 9,100 by the end of 10 days. The temperature throughout the hospital stay was normal except for the 6th and 19th days, when it rose to 100.4° and 100.8°, respectively. On the 7th day, the number of white cells in the urine increased to 30 to 40 per high-powered field. Thereafter, pyuria persisted, with 12 to 20 white cells per high-powered field in catheterized specimens. Simultaneously with the recurrence of pyuria, nonhemolytic *S. aureus* was cultured from the urine; this organism was isolated a number of times but disappeared simultaneously with the pyuria after treatment with penicillin.

Because of the recurrent pyelonephritis, the patient was referred to another hospital for complete study of the renal tract.

Case 2 A 4-month-old boy, was admitted because of convulsions of 24 hours duration (fig 88) The present illness began 10 days prior to admission, when the patient developed a mild upper respiratory infection, which had an uneventful course until the 4th day, when the temperature rose to 103°F. The patient did not appear particularly ill, but on the next day the temperature was 104°F Examination by a physician at that time revealed nothing of note, and treatment with an antipyretic led to a fall in temperature to 100°F for 24 hours The next day the temperature was 103°F and the patient was given one of the sulfonamide drugs; this produced a mod-

A febrile episode on the 26th day was probably related to an upper-respiratory infection, since redness of the pharynx and a watery nasal discharge were present at this time.

Type B *H. influenzae* was isolated from the throat in pure culture several times after recovery from the influenzal meningitis. The patient was observed in the hospital for 2 weeks following the cessation of penicillin treatment and showed no residua or sequelae of the meningeal infections. This part of the course of the illness is not included in figure 88.

Case 3. A 10-month-old girl (fig. 89) became ill 2 days prior to admission, when she cried much more than normally and became markedly drowsy. On the day before entry, she began to vomit, appeared pale, was unusually

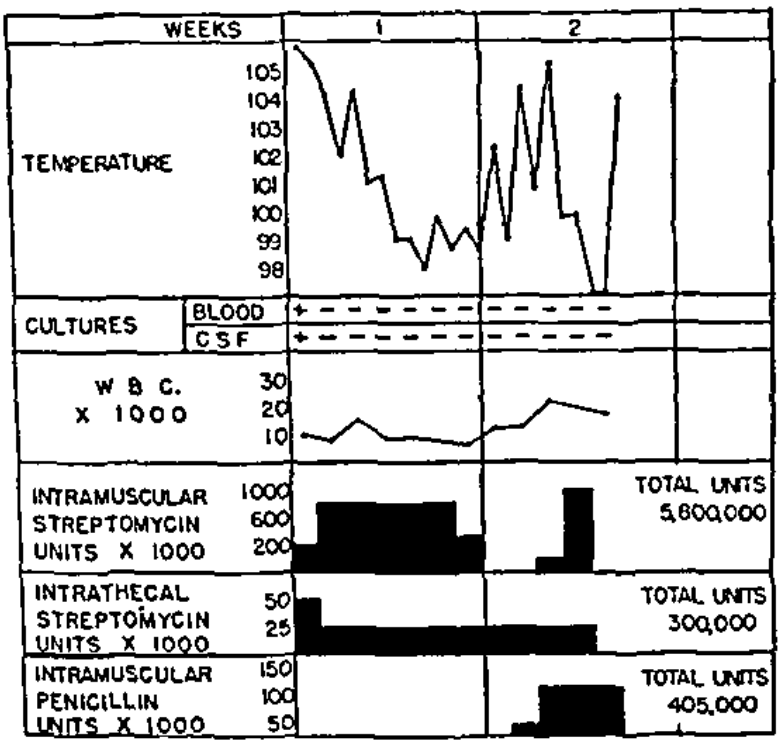

FIG 89 Chart illustrating a complicating *S aureus* pneumonia during recovery from *H influenzae* meningitis and bacteremia (10).

warm, and slept almost constantly. Later in the day, when stiffness of the neck and a temperature of 103°F were noted, the patient was referred to this hospital

Physical examination revealed a well-developed, well-nourished girl who was extremely restless, breathed rapidly, and emitted frequent high-pitched cries The temperature was 105.6°F, the pulse 150, and the respirations 44 She reacted readily to painful stimuli. The anterior fontanelle was normal. There was an internal strabismus of both eyes. The neck and back were stiff Kernig's sign was negative. There was a small area of consolidation in the midportion of the right lung posteriorly. The remainder of the physical examination was within normal limits.

purulent exudate, and the mucous membrane was reddened. The pharynx was slightly reddened but otherwise normal. The neck and back were extremely rigid. Kernig's sign was markedly positive bilaterally. The examination was otherwise within normal limits.

On the patient's admission the urine was normal except for a slight amount of albumin. The white-cell count was 8,500, with 46 per cent neutrophils, 49 per cent lymphocytes, 3 per cent monocytes, and 2 per cent basophils. The hemoglobin was 11 gm. The spinal fluid was under increased pressure and contained 36,400 cells per cubic millimeter, 91 per cent of which were polymorphonuclear leucocytes. The sugar was 30 mg/100 cc, and the protein 50 mg. Many gram-negative pleomorphic rods that exhibited capsular swelling with Type B anti-influenza serum were present. Cultures of the spinal fluid, blood, and nose were positive for Type B *H. influenzae*. This organism could not be demonstrated in the pharynx.

Immediately after the etiology of the meningitis had been established, a dose of 20,000 units of streptomycin was administered intrathecally. Twenty-four hours later, drug administration by the intramuscular route in doses of 50,000 units every 3 hours was also started. Therapy was continued by intramuscular injection for 8 days and intrathecally for 5 additional days. During the 2nd, 3rd, and 4th days, because of the patient's failure to improve clinically, intraspinal injections of 20,000 units of streptomycin were given every 12 hours. The spinal fluid and blood cultures became negative for *H. influenzae* within 24 hours after therapy was instituted. The convulsions, twitchings, stiff neck and positive Kernig's sign disappeared after 11 days of treatment.

On the 8th day, the temperature rose to 104.6°F. Blood and spinal fluid cultures, which had been made daily, showed a coagulase-positive hemolytic *S. aureus* continually from the 7th day onward, but because the organisms grew very slowly and the first three cultures were considered to be possible contaminants, the diagnosis of staphylococcal meningitis and bacteremia was not made until 1 week after onset. *S. aureus* was present in the nose in pure culture on the 8th day. In spite of the new infection of the meninges, the clinical condition was improving rapidly except for the appearance of a scarlatiniform rash over the entire body from the 10th to the 12th day.

Because spinal-fluid and blood cultures were persistently positive for *S. aureus*, penicillin was given intrathecally, 25,000 units every 24 hours, and intramuscularly, 15,000 units every 3 hours, for the first 8 days, when the amount administered by each route was doubled because spinal fluid and blood cultures still revealed the causative organism. Following the increase in dose of the antibiotic agent, these cultures rapidly became negative.

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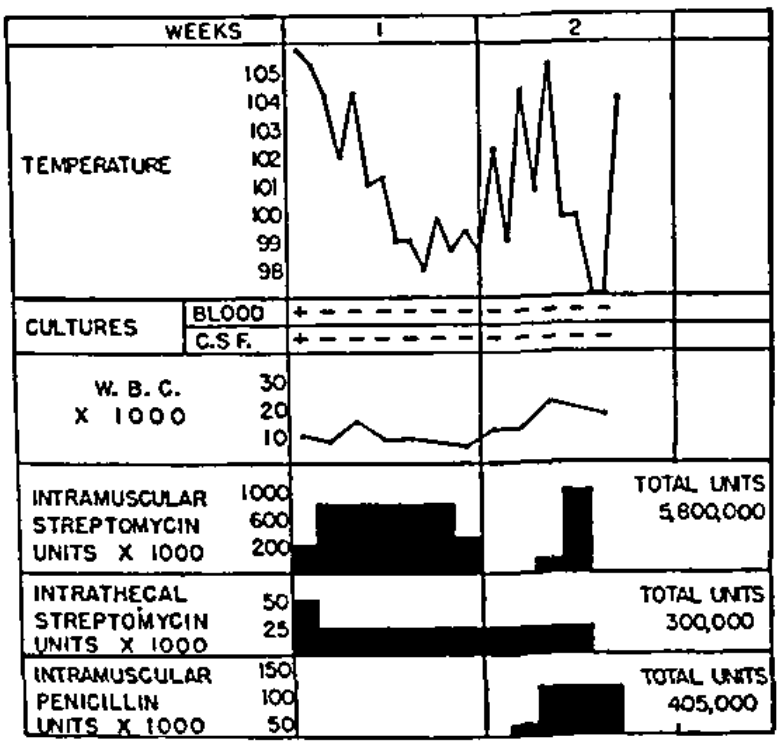

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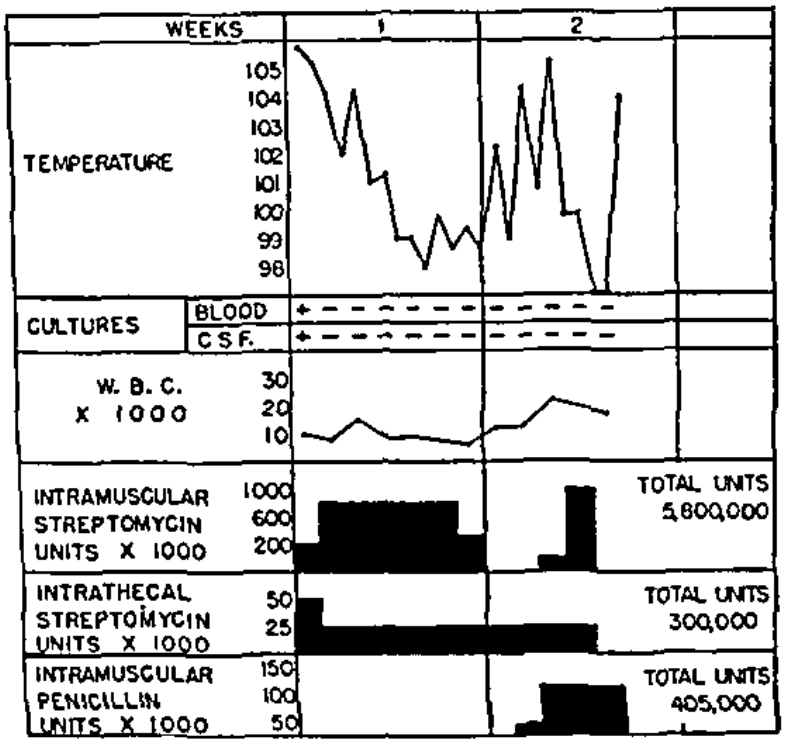

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Physical examination revealed a well-developed, well-nourished girl who was extremely restless, breathed rapidly, and emitted frequent high-pitched cries. The temperature was 105.6°F, the pulse 150, and the respirations 44. She reacted readily to painful stimuli. The anterior fontanelle was normal. There was an internal strabismus of both eyes. The neck and back were stiff. Kernig's sign was negative. There was a small area of consolidation in the midportion of the right lung posteriorly. The remainder of the physical examination was within normal limits.

purulent exudate, and the mucous membrane was reddened. The pharynx was slightly reddened but otherwise normal. The neck and back were extremely rigid. Kernig's sign was markedly positive bilaterally. The examination was otherwise within normal limits.

On the patient's admission the urine was normal except for a slight amount of albumin. The white-cell count was 8,500, with 46 per cent neutrophils, 49 per cent lymphocytes, 3 per cent monocytes, and 2 per cent basophils. The hemoglobin was 11 gm. The spinal fluid was under increased pressure and contained 36,400 cells per cubic millimeter, 91 per cent of which were polymorphonuclear leucocytes. The sugar was 30 mg/100 cc, and the protein 50 mg. Many gram-negative pleomorphic rods that exhibited capsular swelling with Type B anti-influenza serum were present. Cultures of the spinal fluid, blood, and nose were positive for Type B *H. influenzae*. This organism could not be demonstrated in the pharynx.

Immediately after the etiology of the meningitis had been established, a dose of 20,000 units of streptomycin was administered intrathecally. Twenty-four hours later, drug administration by the intramuscular route in doses of 50,000 units every 3 hours was also started. Therapy was continued by intramuscular injection for 8 days and intrathecally for 5 additional days. During the 2nd, 3rd, and 4th days, because of the patient's failure to improve clinically, intraspinal injections of 20,000 units of streptomycin were given every 12 hours. The spinal fluid and blood cultures became negative for *H. influenzae* within 24 hours after therapy was instituted. The convulsions, twitchings, stiff neck and positive Kernig's sign disappeared after 11 days of treatment.

On the 8th day, the temperature rose to 104.6°F. Blood and spinal fluid cultures, which had been made daily, showed a coagulase-positive hemolytic *S. aureus* continually from the 7th day onward, but because the organisms grew very slowly and the first three cultures were considered to be possible contaminants, the diagnosis of staphylococcal meningitis and bacteremia was not made until 1 week after onset. *S. aureus* was present in the nose in pure culture on the 8th day. In spite of the new infection of the meninges, the clinical condition was improving rapidly except for the appearance of a scarlatiniform rash over the entire body from the 10th to the 12th day.

Because spinal-fluid and blood cultures were persistently positive for *S. aureus*, penicillin was given intrathecally, 25,000 units every 24 hours, and intramuscularly, 15,000 units every 3 hours, for the first 8 days, when the amount administered by each route was doubled because spinal fluid and blood cultures still revealed the causative organism. Following the increase in dose of the antibiotic agent, these cultures rapidly became negative.

clear. In two of the patients there was a remarkable change in the bacterial flora in the nose and throat before the new disease developed. Organisms that were apparently present in such small numbers that they were not detected early in the course of treatment increased in number after administration of streptomycin and invaded the tissues. That the new infections were not merely the result of numerical increase of one of the normal inhabitants of the nose and throat is evidenced by the fact that changes in the nasopharyngeal flora following the use of streptomycin were frequently observed without a resultant new infection.

It is possible that in some persons, a high degree of bacterial antagonism exists in areas like the nasopharynx and that certain groups of bacteria are kept in check by others. So long as this normal relationship is not disturbed, the numbers and invasive ability of some of the organisms may be kept below a critical level; when, however, some of the bacteria are removed as a result of contact with an antibiotic agent of high specificity, those organisms that are unaffected by the drug increase sharply in number and possibly in virulence. This phenomenon may be entirely due to an increase in numbers of bacteria if the microorganisms are of sufficiently high virulence. Age, the presence of chronic infection or other debilitating conditions, and preceding chronic or acute pulmonary disease are important factors in increasing the susceptibility to new infections during antibiotic therapy. The type of organisms normally present in the pharynx is dependent to some degree on the general distribution of various bacteria in the population during certain seasons of the year. This will determine, in part, the organism that produces a new infection during the course of treatment with a specific antibacterial agent; for example, in summer, the carrier rate for *H. influenzae* is low and the chance that invasion by this organism will occur as a result of penicillin treatment is less than it is in winter. As *S. aureus* is present in the pharynges of most persons at all times, the seasonal factor probably plays only a minor role in production of disease by this organism in patients who receive streptomycin.

Comments

The spontaneous occurrence of new infections due to nonsusceptible organisms during the course of streptomycin therapy raises the question of the use of this drug in instances where the exact bacteriologic diagnosis is unknown, because patients may be exposed to the added danger of superimposed bacterial diseases without any benefit to the primary process. For example, the treatment of virus infections with streptomycin may be dangerous because this agent has no effect on the primary disease and may allow organisms that are normally present on various tissues and are not

The white-cell count on admission was 10,850, with 70 per cent neutrophils, 29 per cent lymphocytes, and 1 per cent monocytes. The hemoglobin was 12.3 gm. The spinal fluid was under increased pressure and contained 18,250 cells per cubic millimeter, 80 per cent of which were polymorphonuclear leucocytes. The protein was 114 mg/100 cc, and the sugar 26 mg. Direct examination revealed gram-negative pleomorphic rods that gave capsular swelling with Type B anti-influenza serum. The blood culture revealed Type B *H. influenzae*. Nose and throat cultures were negative for *H. influenzae*.

Treatment with streptomycin was started immediately after admission. A dose of 100,000 units was given intramuscularly every 3 hours for 5½ days. After 2 days without treatment, the drug was again given and 1,200,000 units was administered in the next 30 hours. Fifty thousand units of the antibiotic agent was instilled into the spinal canal on admission, and 25,000 units was given every 24 hours thereafter for the next 10 days.

The blood and spinal-fluid cultures revealed no organisms 24 hours after the beginning of treatment, and none were recovered during the remainder of the course. The clinical condition improved somewhat in that the temperature reached a normal level on the 5th day, but coma and twitchings persisted. On the 5th day, a pure culture of *S aureus* appeared in the nose and throat, and this organism was isolated every day thereafter. On the 8th day, the temperature suddenly rose to 102°F, and it remained at high levels for the rest of the course. Physical examination at that time revealed no abnormalities, but on the following day moist, crackling rales were heard throughout both lung fields and x-ray examination revealed diffuse bilateral bronchopneumonia. Since it was thought that this was probably due to *S. aureus*, the patient was treated with penicillin 15,000, units being given intramuscularly every 3 hours for the next four days. The patient remained in coma, convulsions became more frequent and severe, the temperature continued to be elevated, and death occurred on the 12th day. Blood cultures grew out *S. aureus* during the last 2 days of life.

Autopsy revealed that the brain was within normal limits except for two small plaques of fibrin, one on each of the cerebral hemispheres; bacteriologic studies were negative The lungs showed a diffuse confluent bronchopneumonia from which a coagulase-positive hemolytic *S. aureus* was isolated.

Cause of development

The mechanism of the development of the type of infection described in the individuals whose clinical histories are presented above is not at all

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susceptible to its activity to grow profusely and invade. The occurrence of this type of secondary infection is a strong argument for limiting use of any of the antibiotic agents to those cases in which bacterial disease is proved by isolation of the causative agent or to those in which the possibility of bacterial infection is very strong.

Although it might appear that the availability of such agents as streptomycin and penicillin has reduced the necessity for careful bacteriologic studies in patients with infectious diseases, the exact opposite is the case The highly specific antibacterial activity of these drugs necessitates exact identification of the causative agents of the infections for which they are used, and the need for careful bacteriologic study is greater now than it was prior to the advent of the antibiotic substances. This applies not only to the period of the disease before treatment is started but also to the time during which therapy is being given. Otherwise, the manifestations of new infections of the type described in the cases reported above might be misinterpreted as due to failure of the original disease to respond to the drug being used. Frequent bacteriologic examination of the blood and of the nose and throat of patients who are being treated with an antibiotic agent, even though the patients seem to be progressing well, is to be highly recommended because a marked increase in numbers of an organism in the nasopharynx frequently precedes, by at least 24 hours, invasion of the tissues by the organism. The discovery of a preponderance of *H. influenzae* or *S. aureus* in the nasopharyngeal flora during the course of penicillin or streptomycin treatment should put the physician on guard for a complicating infection due to either of these organisms, particularly if the patient is a young child and has a pulmonary infection.

Since new infections may occur spontaneously during administration of penicillin or streptomycin, the question may be raised whether or not these two agents should be given simultaneously to patients who are particularly susceptible to such an event, namely, the very young or very old or those with chronic debilitating disease. The combined use of the antibiotic drugs in a shotgun fashion with the implication that bacteriologic examination is then not necessary can only be decried. Although each of these agents is, on the whole, nontoxic, certain patients may become sensitized to the point where these antibiotics cannot be used. Treatment by a combination of both drugs with an untoward sensitizing reaction may preclude use of either agent some time later in the patient's life when his survival may depend on the drug To advocate employment of a combination of penicillin and streptomycin would result in even greater misuse of these drugs than that to which they are at present subjected, and would only further their use in many diseases in which there is no infectious basis.

Section IV

MISCELLANEOUS USES OF STREPTOMYCIN

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CHAPTER 42

USE OF STREPTOMYCIN AND OTHER ANTIBACTERIAL AGENTS FOR RECOVERY OR ISOLATION OF VIRUSES

Selective media have long served the bacteriologist in the isolation of certain microorganisms from mixed flora. Subject to overgrowth or inhibition by the less fastidious and more vigorous inhabitants of the environment, certain microbes were cultivated successfully when dyes and other agents selectively inimical to the unwanted bacteria were incorporated into the culture medium.

The virologist, confronted with the task of recovering viruses from admixtures with bacteria, relied chiefly for years on the principle of differential filtration. However, the purely physical difficulties inherent in the use of filters of different composition and porosity have limited filtration as a device for the separation of viruses from mixtures with less minute contaminants. The availability of suitable laboratory animals, susceptible to a certain virus, sometimes permitted isolation of the virus from communities of microbes of low or indifferent pathogenicity Recognition of the potentialities of sulfonamide therapy against bacterial infection soon emphasized the refractivity of viruses as a group to these compounds and suggested their use in a selective role. Certain factors, particularly toxicity for the host organism and development of drug-resistant variant bacteria, however, have curtailed the applicability of various sulfa drugs for eliminating bacterial contamination from virus-bearing materials

Penicillin, a forerunner of the new era of antibiotic therapy, soon drew consideration for possible use in freeing viruses and virus-like agents of contaminating microbes. Certain deficiencies of this agent for the purpose intended became apparent early (22, 49) and prompted efforts to complement or abet activity of the drug by the addition of sulfonamides and other compounds (26) With the definition of additional antibiotic substances, several of these, particularly streptomycin, have been employed separately or in combination. Literature bearing on the subject is reviewed in this chapter with the object of evaluating the merits and limitations of the

Mercurial compounds

Merthiolate, 1:10,000 concentration, employed as a preservative for serum used in Newcastle virus neutralization tests, was apparently not toxic to 8- to 10-day old embryonating eggs injected with 0.1 ml of treated serum-virus mixtures by way of the allantoic chamber (33). Toxicity trials by Brandly *et al.* (3) showed that 0.1 ml of chicken serum containing 0.05 per cent (1:2,000) merthiolate when injected into the allantoic cavity produced a lethal effect in a high proportion of 10- to 12-day chicken embryos. However, 0.02 per cent (1:5,000) and 0.01 per cent (1:10,000) dilutions in serum were without apparent effect on the embryo. Hammon and Reeves (19), in similar tests of sera for St. Louis and Western equine encephalitis virus neutralization, found that merthiolate in concentrations of 1:10,000 was not injurious when given to white mice intracerebrally in the usual doses.

Other compounds of mercury including mercuric cyanide, 1:10,000, and phenyl mercuric borate, 1:50,000, employed as anticontaminants were likewise found nontoxic to mice when injected intracerebrally with test sera in the concentrations indicated (19).

Para-aminobenzoic acid (PABA), through virtue of the bacteriostatic efficacy of its various analogs, has found application, for its possible differential selectivity, in the isolation of viruses and rickettsiae. In studies on chemotherapy (11, 12, 35) no effect was noted from the injection of as much as 11 mg of PABA into the yolk sac of 7- to 8-day fertile eggs. When fed as 0.5 per cent of the diet, PABA apparently had no effect on mice or on the course of inoculation-induced psittacosis (strain 6 BC).

Sulfonamides

In 1944 Sigurdsson (47) reported that 7- and 12-day chicken embryos would tolerate sodium sulfadiazine in 40-mg quantities when injected into the amnionic sac and upon the serosa.

The undescribed preliminary experiments of Rose *et al* (44) with sulfadiazine did not stipulate any toxic or other injurious effects upon 11-day embryonating eggs or upon chicken embryo tissue cultures

Chemotherapeutic studies on strain 6 BC psittacosis failed to reveal any toxicity of sodium sulfadiazine in 50 mg per cent solutions upon Maitland tissue cultures or 8-day embryonating eggs (11) The latter were injected with 2.5 to 10 mg amounts into the yolk sac Five-tenths per cent of sulfadiazine in the diet of mice for 7 to 14 days was not found to be toxic to the mice employed in psittacosis infection studies (12). *Sulfadiazine, 0.1 ml of* a 5 per cent solution, injected into the amnionic sac of 13-day chicken embryos was without demonstrable effect on the embryo or the course of the

various antibiotic substances for isolating and manipulating a number o viruses. Pertinent data and studies with certain rickettsia, bacteria, an protozoa are also mentioned briefly. Summarization of information o antibiological agents may be accomplished chiefly from the standpoints o (a) host and tissue tolerance; (b) effect on the virus or organism of whic the isolation or recovery is sought; and (c) certain effects on the flora to b controlled or destroyed.

Host and Tissue Tolerance

Possible injurious effects upon the host organism or cells in which th virus or virus-like agent is to be propagated require primary consideration in the search for substances with suitable suppressive activity against un-desired or contaminating organisms. A period of contact between the con-taminated material and relatively high concentrations of the antimicrobial agent would appear to offer certain advantages if suitable dilution can be accomplished prior to inoculation. If, however, the contaminating organ-isms are not killed during this interval, their effective suppression after introduction into the host tissues may not be accomplished. The ideal antibiotic obviously should have the property of being usable in quantities or concentrations that will eliminate or inhibit the contaminants without significant injury to the host and without favoring development of resist-ance or fastness to the suppressant among the population comprising the unwanted flora.

Detergent agents

Zephiran, a mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides, was used in several attempts to free throat washings (13, 27, 28), contaminated allantoic fluids, and sera (19) of bacteria in order to permit isolation of virus agents or serum neutralization tests. Given intranasally to mice in toxicity trials, 0.05 cc of 1:1,000 solution of zephiran in normal saline did not produce pulmonary irritation (27, 28) Quantities of 0.1 ml of a 1:5,000 solution injected into the allantoic cavity of 10-day embryonat-ing chicken eggs were not injurious during the subsequent observational period of 78 hours (28) In other work (13) no reference was made to pos-sible injurious effects of zephiran on the chicken embryo. The maximum concentration of zephiran in the inocula was 1:10,000 (28)

Other detergents used in these studies (27), Tergitol penetrant (sodium alkyl sulfate), Oakite, No 63 (trisodium phosphate compound), and soap (Ivory) were not toxic when administered intranasally to white mice in 0.05 ml of a 1:1,000 concentration in saline (Tergitol) or of a 1:100 emulsion of Oakite or of Ivory soap.

sant effect upon bacteria admixed with viruses. Trials of the first three on the bacterial flora of fluids and cultures harboring *Tr. foetus* (53) did not include determinations of tolerance or toxicity for animal hosts or tissues. Streptothricin introduced on the serosa of 8-day eggs at the rate of 500 units per egg was apparently very toxic, since only 9 per cent of the embryos survived 6 days after treatment (15).

Penicillin

Used alone as an antibacterial agent, penicillin, chiefly as the sodium salt, has not been reported to show toxic or injurious effects upon tissue cultures (11, 39, 44), developing chicken embryos or the extra-embryonic membranes (2, 3, 5, 11, 18, 21, 23, 37, 38, 39, 41, 43, 44, 45, 52), mice (12), monkeys (2), or rabbits (17). The maximum quantities of penicillin introduced were 500 units into roller tube tissue cultures (44), 500 units on the chorio-allantoic membrane of 11-day chicken embryos (43), 4,000 units into the allantoic cavity of 10-day embryonating eggs during a 4-day period (3), 500 units in two doses with a 24-hour interval into the amnionic cavity of eggs incubated 8 days prior to the first injection (11), and 100 units into the yolk sac of 5- to 6-day eggs (2). Mice were given, without apparent ill effect, as much as 112,000 units of penicillin by the subcutaneous route over a 14-day period or by providing 1,000 units of penicillin per milliliter of drinking water for continuous consumption. Monkeys were injected in the parotid duct with 1 cc of material containing 25 units penicillin (2), and rabbits were injected intramuscularly with 24,000 units over a period of 3 days (17) without any effect.

These maximal amounts of penicillin were contained in sputum (44), chorio-allantoic membrane (43), yolk sac suspensions or tissue culture fluids (11), saliva (2), normal saline or water (12), vaccine lymph (17), or alimentary tract and fecal material (3). Penicillin has been employed also in combination with other drugs and antibiotics in efforts to augment its activity or to overcome apparent deficiencies as an antibacterial agent in relationship to culture or isolation of viruses, rickettsiae, and certain protozoa

Johnson *et al.* (25), in attempts to destroy bacteria which contaminated cultures of *Tr. vaginalis*, added tyrothricin and sulfathiazole (quantities not given), but found neither agent advantageous over 500 to 1,000 units of penicillin per milliliter. The toxicity of the combination was not tested in embryonating eggs or other organisms.

Burnet and Stone (5), in attempts to isolate influenza virus from throat washings, injected with no injurious effects, 0.1 ml of 5 per cent sulfadiazine into the amnionic sac of 13-day chicken embryos followed by 0.1 ml of a throat washing-penicillin mixture containing 10 units of penicillin.

influenza infection (5). This drug, included as 0.1 per cent of the diet for 10 days, was not injurious to mice (42).

Sodium sulfathiazole added as a preservative (0.2 per cent) to sera used in intracerebral virus neutralization tests in mice by Hammon and Reeves (19) became toxic to the mice after the preserved sera had stood for a few days. Zephiran, merthiolate, phenyl mercuric borate and mercuric cyanide in the concentrations employed did not elicit the serotoxic effect (19) Sulfathiazole composing 1 0 per cent of the diet for 10 days was not harmful to mice (42). The maximum tolerated doses of several sulfa compounds as determined by yolk-sac injection of 10-day eggs was as follows: promin, 30 mg; sulfathiazole, 10 mg; promizole, 1 mg; and several sulfones, 0 5 to 1.0 mg per 100 gm of egg (29).

Sulfapyridine, prontosil, proseptasine, and sulfanilamide were employed by Rudd and Burnet (46) in intranasal psittacosis infection experiments in mice. Sulfapyridine in total quantities of 200 mg over a 72-hour period and the other three drugs in 110-mg doses over 30-hour periods were nontoxic by the intranasal route.

The literature dealing with host tolerance to sulfonamides in combination with antibiotics will be reviewed in the later respective sections.

Miscellaneous agents

Patulin introduced daily for 5 days in 0.1-mg amounts upon the serosa of 11-day embryonating eggs previously inoculated with fowl pox had an injurious effect Most of the membranes became necrotic, and a large proportion of the embryos died (43). Patulin was found lethal to 16- to 19-gm Swiss mice when injected intraperitoneally and subcutaneously in 0.25- to 1-mg amounts Mice that received 0.25 mg intravenously survived One per cent buffer solutions of patulin caused conjunctival edema, congestion, and swelling when dropped into the eyes of rabbits. Twelve-day chicken embryos were killed by quantities as small as 0.25 ml of a 1:1,600 dilution into the allantoic chamber and 0 5 ml of 1:800 solution upon the serosa (48).

Propamidine and pentamidine, quantities not stated, tested for their antibacterial activity on unfiltered sputums inoculated into embryonating eggs were reported to be lethal to the 11-day embryos (44)

Phenol added to chicken sera in final concentrations up to 1 per cent had no immediate or delayed adverse effect on 10- to 12-day chicken embryos used for titrating virus neutralization activity. Tests were made after storage at 6 to 8°C for intervals up to 11 months (3). In other work, phenol concentrations of 1 and 2 per cent were not recorded to have an injurious effect on chicken embryos (6, 51).

Antibiotics such as clavicin, gramicidin, actinomycin, and streptothricin have apparently found little experimental usage for their possible suppres-

in 0.2-ml quantities, streptomycin gave no evidence of ill effects on the egg (1). Similar innocuity of this antibiotic was seen when used by us (54).

Effect of Antibiological Agents on the Virus or Isolant

According to present knowledge, differences in response to antibiotic agents among bacteria, rickettsiae, and viruses are associated with fundamental differences in the nature and metabolic activities of these organisms. Substances such as streptomycin appear to interfere with or disrupt certain of the enzyme systems or metabolic processes of bacteria, often with resultant complete inactivation or death. On the other hand, the rickettsiae and viruses as a group, lacking such systems of metabolism and multiplication, are inhibited or retarded only temporarily, if at all. An indirect effect, however, may occur whereby the toxicity of the antagonistic agent may influence the host cells or system adversely. The simpler microbes and viruses, more intimately parasitic as evidenced by required intracellular habitat, are frequently unaffected by substances that may interfere with the largely extracellular mechanisms of the more complex organisms. The differential activity of penicillin and streptomycin for gram-positive and gram-negative bacteria appears to be reflected in differences in effect and response among larger and smaller viruses; for example, psittacosis virus is susceptible to penicillin, whereas the influenza virus is not. Certain antibiotic or other agents now known or probably to be found in the future may thus be useful in separating mixtures of one or more viruses or rickettsiae, as well as in recovering viruses, rickettsiae, and bacteria from admixtures with one another.

The natural occurrence or development of resistance or fastness among susceptible species of organisms to certain antibiological agents obviously bears on the usefulness of such methods of segregation of various microbes.

The recent literature on the subject of isolating certain organisms by utilizing selective agents deals chiefly with the effect on unknown and diverse flora with only limited consideration of specific organisms which may have shown a greater or lesser degree of fastness In contemplating the use of one or a mixture of antibiotic agents or drugs, the quantitative factor can seldom be disregarded with respect to possible specific inhibitory effect upon the virus or organism whose isolation is sought. In the following section, attention is drawn to efforts that have been made to determine and evaluate the effects of various antibiological agents or materials on the parasitic virus or organism.

Viruses

The possibility that any agent which would serve to inhibit or inactivate bacteria might have an inimical effect on viruses in the same environment received attention early.

Beveridge *et al.* (2) attempted direct isolation of mumps virus from the saliva of man by first injecting the yolk sac of 5- to 6-day embryonating eggs with 0.4 ml of 5 per cent sulfadiazine followed by introductions of centrifuged saliva that had been treated with penicillin to make a final concentration of 300 units. Isolation of virus was accomplished in three of seven cases, and no evidence of injurious effect of either drug or of the combination of the two was recorded.

When penicillin was used together with streptomycin, no ill effects were noted on chicken embryos or on the extra-embryonic membranes (1, 8, 23, 30, 32, 45, 52). The range of incubation times prior to injection was from 7 (52) to 14 (23) days. The sites of introduction included allantoic (1, 45, 50, 52) and amnionic (23, 30, 40) chambers. The maximal quantity injected was approximately 4,800 units streptomycin and 2,000 units penicillin per egg (8). The antibiotic-treated inocula represented mumps-infected allantoic fluid (52), normal allantoic fluid that had been used to wash air from a poultry house harboring chickens infected with Newcastle disease (8), human nose and throat washings (32), sputums (32, 45), lung suspensions (32), saliva (30), chicken respiratory exudates (1), and stools (23).

Streptomycin

Since streptomycin has become available and its efficacious properties have become better known, it has found considerable usage either alone or in combination with penicillin and other agents in attempts to eliminate undesirable bacteria.

Studies with streptomycin as an antibacterial agent yielded no reports of observable ill effects on embryonating eggs in the concentrations or quantities employed (11, 31, 35, 45, 49, 52). The test eggs had been subjected to prior incubation for 7 (35) to 11 (31, 45) days. The streptomycin-bearing inocula were introduced on the serosa (49), yolk (11, 35) and allantoic (31, 45, 52) chambers, and represented tissue culture fluids (11), allantoic fluid (11, 52), yolk sac material (35), throat washings (31), sputums (45), and feces (31). The total quantity of streptomycin used per egg ranged from 250 (11) to approximately 5,200 (31) units. Mice were given 28,000 units at the rate of 200 units a day for 14 days (12) and 5 units ml was employed in tissue cultures (11). Emmart (15), however, reported 68 per cent survival of 8-day embryos treated with 1,000 units streptomycin on the chorio-allantoic membrane

A mixture containing streptomycin (4,000 units), tyrothricin (0.08 mg), and sulfadiazine (2 mg/ml) as defined by Eddie (14) has been used in attempts to isolate Newcastle virus from material contaminated with bacteria. Admixed in equal proportions with respiratory tract exudates of [illegible] injected into the allantoic cavity of 10-day embryonating eggs

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Viruses

The possibility that any agent which would serve to inhibit or inactivate bacteria might have an inimical effect on viruses in the same environment received attention early.

The work of Krueger and associates (27, 28) dealt with various detergents as antibacterial agents in contaminated allantoic fluids, saliva, and mouse lungs which carried influenza A or B viruses. Concentrations of zephiran twenty times as great as that required to eliminate the bacteria within 20 minutes at room temperature were without harmful effect on the virus strains used. Teigitol penetrant, No. 7, 1:2,000 in 5 per cent infected mouse lung reduced the activity of the influenza viruses as indicated by increased survival time of the infected mice from 4 to 6 days to 10 or more. Oakite, No. 63, 1:100 had some inactivating effect on the type B virus but not on the A type. Ivory soap 1:100 rendered the 5 per cent suspension of B virus partly inactive and the A virus completely so in 40 minutes. Other workers (13) reported that throat washings treated with zephiran as described (27, 28) showed subsequent overgrowth of bacteria; hence, the possible effect of zephirin on any influenza virus which may have been present could not be evaluated

Subsequent attempts by Hirst (20) to free throat washings of contaminating bacteria by use of various concentrations of gramicidin and sapamine failed, since concentrations that injured the bacteria also injured the virus

Sulfadiazine in doses up to 40 mg failed to alter the influenza A virus infectivity end-points or the hemagglutinin titers of the chicken allantoic fluids (47). Rose *et al.* (44) were unable to evaluate the possible effect of sulfadiazine on virus in influenza-suspected sputums, since preliminary experiments indicated that even in high concentrations the drug was inadequate as a bacteriostatic agent. Burnet and Stone likewise were unable to ascertain any anti-influenza virus effect because sodium sulfadiazine failed to control bacterial contamination.

Observable injurious effects of sodium penicillin on influenza viruses A and B in various menstrua were not seen in isolation or infectivity trials on tissue cultures, embryonating eggs, and mice (5, 21, 32, 44). The range of penicillin concentrations was from 100 (5) to 1,000 (44) Oxford units per milliliter

Streptomycin did not inhibit the growth of PR8, Olsen and Lee, strains of influenza virus injected into the allantoic chamber of 11- to 13-day embryonating chicken eggs. Massive quantities, 12,000 units, were given over a 24-hour period (16). As much streptomycin as 2,500 units ml, or 5,200 units per egg, had no adverse effects on a strain of influenza A virus (31)

When influenza-infected nose and throat washings and mouse lungs were treated with a combination of 1,000 units/ml of streptomycin and 500 units/ml of penicillin *there was no apparent inhibition of growth of types* A and B influenza virus (32).

Newcastle disease virus, which has some properties in common with influenza (4), has not been found to be injured or altered by merthiolate,

1:5,000 in chicken serum (3); by phenol, 1:100 in chicken serum (3); by sodium penicillin, 4,000 units or less per embryonating egg in allantoic fluids (3, 41), intestinal content (3), or serum (41)

Mixtures of penicillin and streptomycin, 1,000 units each per milliliter, used for isolating Newcastle virus from exudates and tissue suspensions, appeared to have no more effect on the recovery of the virus than did filtration (1). Similar results were obtained with the mixture of Eddie (14). In evaluating the suitability of streptomycin for Newcastle virus isolations from various tissues of chickens, Thompson (50) found that 25 mg (25,000 units)/ml, a concentration of sufficiently low toxicity to the embryonating egg, did not reduce the titer of the 11914 strain of virus in tissues at ice-box temperature, but at room temperature a slight drop in virus titer occurred after 4 days.

Newcastle virus was isolated from the air of infected poultry houses by drawing the air through normal allantoic fluids and, after treatment with 24,000 units streptomycin and 10,000 units penicillin/ml, inoculation into 11-day eggs (8).

The DEI strain of fowl plague (fowl pest) virus (38) appeared to be unaltered by the same concentrations of penicillin, phenol, and merthiolate employed in studies with Newcastle disease virus (3).

Mumps virus was isolated directly from saliva introduced into 5- to 10-day embryonating eggs or indirectly through monkeys by the use of penicillin, 100 to 300 units per egg, or by combining penicillin with the injection of 0.4 cc of 5 per cent sulfadiazine into the yolk chamber (18)

Mumps virus growth in 7-day embryonating eggs was not influenced by 500 units/ml of penicillin or by 100 μg/ml of streptomycin or by these antibiotics combined in the same concentrations. Preinoculation storage was held at 4°C for as long as 20 days (52) Addition of 250 units of penicillin and 2,500 units of streptomycin permitted isolation of mumps virus from human saliva and monkey parotid (30).

An evaluation of the effects of several antibacterial agents may be made from attempts to isolate a number of neurotropic and pneumotropic viruses cultured in Rivers-Li medium with Tyrode solution or in the chick embryo. No effects were observed on St. Louis and Eastern equine encephalitis viruses with the maximum concentrations of penicillin used, 10 units/ml. Meningopneumonitis virus and psittacosis 6 BC growth were, however, depressed considerably by high concentrations of penicillin, 200 units/ml (39)

The St Louis and Western encephalitis viruses, as well as the Lansing poliomyelitis virus, were not affected by preinjection admixture with sera preserved with 1:10,000 zephiran, 1:10,000 merthiolate or mercuric cyanide, 1:50,000 phenyl mercuric borate, or 1:500 sodium sulfathiazole (19).

Sulfathiazole or sulfadiazine used as 0.1 per cent of the feed of mice given

The work of Krueger and associates (27, 28) dealt with various detergents as antibacterial agents in contaminated allantoic fluids, saliva, and mouse lungs which carried influenza A or B viruses. Concentrations of zephiran twenty times as great as that required to eliminate the bacteria within 20 minutes at room temperature were without harmful effect on the virus strains used. Tergitol penetrant, No. 7, 1:2,000 in 5 per cent infected mouse lung reduced the activity of the influenza viruses as indicated by increased survival time of the infected mice from 4 to 6 days to 10 or more. Oakite, No. 63, 1:100 had some inactivating effect on the type B virus but not on the A type. Ivory soap 1:100 rendered the 5 per cent suspension of B virus partly inactive and the A virus completely so in 40 minutes. Other workers (13) reported that throat washings treated with zephiran as described (27, 28) showed subsequent overgrowth of bacteria; hence, the possible effect of zephirin on any influenza virus which may have been present could not be evaluated.

Subsequent attempts by Hirst (20) to free throat washings of contaminating bacteria by use of various concentrations of gramicidin and sapamine failed, since concentrations that injured the bacteria also injured the virus.

Sulfadiazine in doses up to 40 mg failed to alter the influenza A virus infectivity end-points or the hemagglutinin titers of the chicken allantoic fluids (47). Rose *et al.* (44) were unable to evaluate the possible effect of sulfadiazine on virus in influenza-suspected sputums, since preliminary experiments indicated that even in high concentrations the drug was inadequate as a bacteriostatic agent. Burnet and Stone likewise were unable to ascertain any anti-influenza virus effect because sodium sulfadiazine failed to control bacterial contamination.

Observable injurious effects of sodium penicillin on influenza viruses A and B in various menstrua were not seen in isolation or infectivity trials on tissue cultures, embryonating eggs, and mice (5, 21, 32, 44). The range of penicillin concentrations was from 100 (5) to 1,000 (44) Oxford units per milliliter.

Streptomycin did not inhibit the growth of PR8, Olsen and Lee, strains of influenza virus injected into the allantoic chamber of 11- to 13-day embryonating chicken eggs. Massive quantities, 12,000 units, were given over a 24-hour period (16). As much streptomycin as 2,500 units ml, or 5,200 units per egg, had no adverse effects on a strain of influenza A virus (31).

When influenza-infected nose and throat washings and mouse lungs were treated with a combination of 1,000 units/ml of streptomycin and 500 units/ml of penicillin there was no apparent inhibition of growth of types A and B influenza virus (32).

Newcastle disease virus, which has some properties in common with influenza (4), has not been found to be injured or altered by merthiolate,

yolk sac or 0.5 per cent via the diet, on 6 BC psittacosis infection in embryonating eggs or in mice, respectively.

Rickettsiae

Efforts to isolate rickettsiae or to treat infections with these agents have yielded data on the susceptibility of these microbes to certain antibiotic and other substances. The active growth phase of typhus rickettsiae in the yolk sac of embryonating eggs was inhibited by introduction of penicillin (18).

Streptomycin, 0.5 to 2.0 mg per egg, or para-aminobenzoic acid, 5 5 and 11 mg, was injected into the yolk sac of 7-day eggs 2 hours prior to infection with *R. mooseri*, Wilmington strain, *R. prowazeki*, Breinl strain, or *R. orientalis*, Karp strain. PABA definitely inhibited growth of all the rickettsiae, *R. prowazeki* being the most sensitive. This strain was most susceptible, also, to streptomycin, the other two species not being affected significantly by 2 mg or less, which slowed the growth of *R. prowazeki* (35). Q fever infection (*R. burneti*) in mice was retarded by 0.5-mg doses of streptomycin, but even 10-mg doses were not rickettsiocidal (24).

Pleuropneumonia-like organisms

Recovery of pleuropneumonia-like organisms from pathological specimens with the aid of penicillin (10), as well as the isolation of such pleomorphic forms from cultures of *H. influenzae* (9), warrants mention here because of the small size and the metabolic requirements of these bacteria and also because of possible selective mechanisms involved.

Protozoa

Certain protozoa have been found less susceptible than various bacteria to certain antibacterial substances, and, hence, the possible use of agents of the latter class for isolation purposes has been investigated. Exposure of specimens containing *Tr. vaginalis* to 5,000 to 10,000 units penicillin per 10 ml medium for 60 hours was adequate to destroy contaminating bacteria and ensure isolation of the protozoa. The addition of tyrothricin produced a lethal effect on the trichomonads (25) Concentrations of clavacin, gramicidin, and actinomycin sufficient to suppress various common bacteria were too toxic to *Tr. foetus* to permit their utilization for freeing *Tr. foetus* cultures of such organisms (53). Penicillin, 100 units/ml, was not toxic to *Tr foetus* Streptomycin in equal concentrations also was tolerated well by the trichomonads and largely eliminated the penicillin-resistant bacteria. Similar results were obtained with *Tr. vaginalis* cultures by 10 hours' exposure to 10 units penicillin and 25 units/ml of streptomycin (40). In attempts to free deliberately contaminated *Tr. foetus* cultures, the protozoa

100 LD50 intranasal doses of meningopneumonitis or mouse pneumonitis viruses did not alter the induced disease (42).

Vaccinia virus strains, BH and CVII, grown on Rivers-Li medium and on the serosa of 14-day embryonating eggs, showed no response to 10 units/ml and 500 units of penicillin, respectively, the maximum concentration employed (39). In the preparation of vaccine lymph, vaccinia virus appeared more sensitive to inactivation by penicillin than by glycerin. The quantities of penicillin used, 100 units/ml, killed the virus in 3 days at room and ice-box temperatures. Good results were reported from local and intramuscular application of penicillin (with ointment containing 500 units/gm and 1,000 units every 3 hours for 3 days, respectively) only when treatment was initiated very early in the course of experimental vaccinia infection in rabbits (17).

Fowl pox virus infection of the chorio-allantoic membrane of 11-day embryonating eggs was not modified by instillation of 500 units of penicillin on the membrane during a 5-day period (43). The effect of 0.1 mg of patulin on fowl pox infection could not be evaluated because of injury to the tissues or lethal effect on the embryo.

Herpes virus was isolated in 11-day eggs from a sample of sputum treated with 500 to 1,000 units/ml of penicillin (19).

Psittacosis (strains B and P) infection in mice was not influenced by sulfapyridine (200 mg during a 72-hour period) or by administration of this drug, prontosil, proseptamine, or sulfanilamide (110 mg in 30 hours) (46). In contrast, growth and infection by the 6 BC strain of psittacosis virus (11) was greatly inhibited, although the virus was not killed by 50 mg per cent concentrations of sodium sulfadiazine in roller tube tissue cultures (11) and 2.5 to 10 mg injected into the yolk sac. Sulfadiazine blood levels of 8 to 16 mg per cent obtained by feeding also protected against fatal infection from moderate exposure to 6 BC strain (12).

Psittacosis virus, 6 BC, was also inhibited from growing in roller tube cultures by 5 units/ml, as indicated by a rise in virus titer after penicillin treatment of the culture was withheld (11). Penicillin in doses of 8,000 units a day subcutaneously for 14 consecutive days or as 1,000 units/ml of drinking water reduced the rate of infection and mortality from the 6 BC strain in mice (12). Penicillin combined with sulfadiazine therapy produced effects no different from those with either alone (12).

Streptomycin in concentrations of 5 units/ml of tissue culture fluid or in 250-unit doses injected into eggs had no appreciable effect on the 6 BC virus (11). Administration of 2,000 units a day for 14 days by the subcutaneous route likewise did not change the course, mortality, or carrier rate in mice infected with this strain (12) The work of Early and Morgan (11, 12) failed to show any effect of para-aminobenzoic acid, 4-mg doses into the

lated into the amnionic sac. This method was, however, unsuccessful when yolk-sac inoculation was attempted. Reisolation of virus from nasal washings of infected ferrets was possible also by use of these two bacteriostatic agents (5).

Addition of 2,500 units of streptomycin to mixtures of allantoic fluid infected with influenza A and normal throat washings resulted in survival of four of six 11-day-old embryos inoculated into the allantoic chamber. Six embryos injected with untreated washings died (31).

When nose and throat washings from the 1945 influenza B epidemic were treated with penicillin, 500 units/ml, and injected into embryonating eggs, two-thirds of the embryos died. When 1,000 units of streptomycin and 500 units of penicillin per milliliter were employed, the embryo mortality was reduced to less than 10 per cent. The combined antibiotics rendered bacterial contamination infrequent and when used in mouse lung suspensions were effective in reducing bacterial pneumonias during rapid serial passage of influenza virus in mice. *Ps. aeruginosa* in mouse lung suspension was resistant to streptomycin (32).

Saliva and sputum

After preliminary trials with sulfadiazine in high concentrations indicated the inadequacy of the drug as a bacteriostatic agent for sputums, Rose *et al.* (44) added penicillin at the rate of 500 to 1,000 units/ml. After inoculations of fifty-five sputum samples, with and without penicillin, into tissue cultures and into the amnionic chamber of 11-day embryonating eggs, 96 3 per cent of the tissue cultures receiving the untreated and 41.8 per cent inoculated with treated sputums showed heavy early bacterial contamination. Forty-nine embryos, or 89.1 per cent, receiving the untreated material died, 47 within 24 hours; whereas 85.5 per cent of those receiving the penicillin-treated material survived for at least 5 days and all were sterile. Most of the bacteria recovered from injected eggs were gram-negative and consisted chiefly of *Proteus*, *Pseudomonas*, and penicillin-resistant staphylococci. To control penicillin-insensitive organisms, Rose *et al.* (45) later employed streptomycin alone, 500 units/ml, or in combination with penicillin, 500 units/ml. Of 120 control embryos, 7 per cent survived, as compared to 39 per cent of those receiving penicillin, 50 per cent of those receiving streptomycin, and 64 per cent of those injected with sputum treated with both agents. The selective effect of penicillin and streptomycin for gram-positive and gram-negative organisms, respectively, was well demonstrated by study of the surviving flora, and it was observed that the two antibiotics may act synergistically.

Penicillin, 250 units/ml, and streptomycin, 2,500 units/ml, added to saliva collected during the acute inflammatory stages of mumps, permitted

were not affected by 1,000 units streptomycin or 10,000 units penicillin, but 80,000-unit quantities of penicillin per milliliter were lethal (34).

Effect on Contaminant or Unwanted Flora

With summation of the information available on the tolerance of the host organism or tissue, as well as the parasite the recovery or isolation of which is sought, evaluation of the possible uses and efficacy of the antibiological substances may be better accomplished. It is to be anticipated that the effects of certain of these substances on a given organism or groups of organisms in known menstrua and environments may not be comparable to those which may be obtained with unknown flora under diverse and uncontrolled conditions. The normal flora of the several body systems or tracts is subject to considerable variation in health and to much greater changes under the impact of disease. Hence, the limitations of one or several antibacterial agents apparently can seldom be closely defined.

It is believed that scrutiny of the literature from the standpoint of effect of antibacterial agents employed for isolation of certain viruses or organisms may be best approached by considering, first, the materials from which recovery or isolation is sought and, second, the effect of the antibacterial substances on known specific bacteria that may be common contaminants in nature. Certain data that tend to establish definite limitations of resistance or susceptibility among bacteria are also pointed out.

Nose and throat washings

Allantoic fluid-normal throat washing mixtures to which zephiran 1:20,000 was added were free of viable bacteria after standing at room temperature for 20 minutes (27). Attempts to demonstrate influenza virus in throat washings by this method gave overgrowth of bacterial contaminants. Although the virus was isolated in one instance of zephiran-treated washings, it was impossible to obtain a bacteria-free culture even after four allantoic passages of zephiran-treated fluids The contaminating streptococcus was finally removed by filtration (13)

Treatment of throat washings from influenza A patients with 125 units penicillin per milliliter prior to amnionic inoculation into embryonating eggs proved more effective than did collodion-membrane filtration in overcoming bacterial contamination The former was likewise more effective than inoculation of ferrets with unfiltered washings In allantoic-sac inoculation, use of relatively higher concentrations of penicillin than for amnionic-sac inoculation was advised (21)

Combination of penicillin, 100 units/ml, and sodium sulfadiazine, 0.1 ml of a 5 per cent solution, for isolation of influenza virus was successful in overcoming bacterial contamination of unfiltered throat washings inocu-

treated with penicillin, the presence of pleuropneumonia-like organisms could be readily demonstrated (10).

Fecal material

Penicillin alone was found sufficiently antibacterial in action to permit isolation in embryonating eggs of Newcastle disease and fowl plague viruses from the intestinal content of chickens (3, 38). The bacteria of 10 per cent stool suspensions of epidemic diarrhea patients, when treated with 25 to 200 units of penicillin per 0.05 ml and inoculated after 30 to 90 minutes into the amnionic sac of 13- to 14-day embryonating chicken eggs, were not inhibited to the extent of preventing death of the embryos. These concentrations of penicillin, however, when supplemented with 5,000 μg per 0.05 ml, permitted sufficient bacterial suppression for survival of 50 per cent of the stool-inoculated embryos (23).

Marked antibacterial effects of streptomycin were exhibited as a result of treating 1:50 suspensions of stools with 2,500 units/ml, leaving them at room temperature for 1 hour, and centrifuging them. Additional streptomycin was then admixed to a total of about 2,500 units per 0.1 ml of inoculum for each egg (31).

Organs and tissue suspensions

The antibacterial activity of streptomycin and penicillin in mouse lung suspensions has been cited (32). Lung suspensions and pooled brain, liver, and spleen suspensions of chickens collected during outbreaks of Newcastle disease were centrifuged and treated with 25 mg of streptomycin per milliliter (50). After standing at room temperature for one-half hour, 13-day embryonating eggs were injected with 0.3 ml intra-allantoically. Only three streptomycin-tolerant organisms were recovered from 258 sample tests, of which forty-five yielded isolates of Newcastle virus.

Penicillin, 100 units/ml, added to vaccine lymph, was distinctly inferior to the conventional 50 per cent glycerin employed for eliminating staphylococci and other bacteria.

Contaminated serum

Addition of 1:5,000 or 1:10,000 merthiolate served to prevent growth of eliminated bacteria from serum used in serum-virus neutralization tests (3, 19, 33, 38). Zephiran or mercuric cyanide, 1:10,000, and phenyl mercuric borate, 1:50,000, were likewise effective for this purpose, but sodium sulfathiazole 1:500 rendered the treated sera toxic to the test mice (19).

Embryonic fluids and tissues

Although virus-infected allantoic and amnionic fluids, extra-embryonic membranes, and the avian embryo itself, in part or entirely, have been used

elimination of bacterial contamination and isolation of the virus in 8-day embryonating eggs (30).

Respiratory tract secretions and exudates

Respiratory tract secretions and exudates, as well as intestinal content and feces, from chickens affected with Newcastle disease (3) or fowl plague (38), were treated with penicillin, 1,000 units/ml, prior to egg inoculation. Seldom was the growth of all bacteria suppressed, but bacteria-free virus could be recovered frequently after inoculation with high dilutions of material which yielded less than 1,000 bacterial colonies per milliliter on plating. Respiratory tract exudates and lungs from chickens suspected to be suffering from Newcastle disease were found to contain a varied flora of bacteria including, *Escherichia, Salmonella, Pasteurella, Proteus,* and *Pseudomonas* (1). Cultures for bacteria were negative following treatment with penicillin and streptomycin, 1,000 units of each per milliliter, and storage at 37°C for 7 hours and at room temperature for 9 additional hours. Similar suppression of bacteria was obtained with the tyrothricin–sodiumsulfadiazine–streptomycin mixture of Eddie. Treatment of poultry house air washings with 10,000 units penicillin and 24,000 units streptomycin and subsequent incubation at 9°C for 4 hours resulted in freeing the material of viable bacteria (8).

Genito-urinary tract secretions and tissues

Treatment of vaginal secretions with 5,000 to 10,000 units of penicillin per milliliter destroyed the bacteria present and allowed cultivation of *Tr. vaginalis* (25) Combinations of penicillin with sulfathiazole or the addition of tyrothricin provided no advantage over penicillin alone.

Various bacteria from vaginal mucus, such as streptococci, *Escherichia, Aerobacter, Proteus,* staphylococci, and *Pseudomonas,* were found lethal to *Tr foetus* in 24 to 48 hours in absence of several antibiotics. Clavacin, gramicidin, and actinomycin in sufficient concentration to control these bacteria were toxic to the trichomonads, but penicillin and streptomycin in concentrations innocuous to these protozoa destroyed all of the bacteria except *S aureus* amd *Ps aeruginosa* It was reported that 1,000 units/ml of the antibiotics was necessary against *Ps aeruginosa* (53). Contaminating *S. aureus* and *Corynebacterium* were eliminated by 100 and 125 units/ml of penicillin, whereas an atypical *C. renales* required 200 to 300 units. A *S. bovis* strain resisted 50,000 units/ml penicillin. Furthermore, 1,000 units of streptomycin failed to eliminate contaminating *E. coli* and *B. subtilis* (34).

Cultures of *Tr. foetus* were also freed of bacteria after 10 hours exposure to 10 units penicillin and 25 units streptomycin per milliliter (40).

When cultures of bacteria from diseased genito-urinary specimens were

lantoic fluid 48 hours after allantoic chamber injection of 2,500 units per milliliter showed persistence of bactericidal concentrations. Only small amounts, however, appeared in the amnionic fluid (31).

The possibility that the tolerance to or response of the host organism, for example, the embryonating egg, to antibacterial agents may be influenced by factors such as route of injection may not be disregarded. Treatment of throat washings of influenza A patients with penicillin did not alter the relatively greater sensitivity to virus infection by the amnionic over the allantoic chamber route (21).

Utilization of antibacterial agents for isolation of viruses and rickettsiae has revealed significant differences between these groups of parasites, as well as among strains of each, in their susceptibility to inhibition or injury. The response of the latter may also be governed by occurrence or nonoccurrence of active growth or multiplication. Much of the work already cited indicates that the rickettsiae are more subject than are the viruses to the inhibitory effects of various antibacterial substances. Although Morgan *et al.* (35) determined that minute quantities of streptomycin would permit growth of certain rickettsiae in egg yolk sacs, they cautioned against the use of streptomycin in attempts to isolate the organisms of murine and epidemic typhus from material contaminated with bacteria. No evidence has been encountered which suggests that similar low concentrations, 2 mg, of this antibiotic will inhibit growth of the classical viruses Viruses such as psittacosis (39), meningopneumonitis (39), and vaccinia (17) may, however, be injured by relatively high concentrations of penicillin which are without effect on other and smaller viruses. Studies with the 6 BC strain of psittacosis virus in eggs and tissue cultures (11) and in mice (12) indicated its refractivity to streptomycin and to para-aminobenzoic acid and its sensitivity to 5 units/ml of penicillin or 50 mg per cent of sodium sulfadiazine. Differences in response of this virus to sulfadiazine in static and actively growing cultures of tissue emphasized that this drug, in the concentrations used, affects the virus only in the active growth phase.

An early example of differences between types of a virus was seen in the greater susceptibility of type B influenza than of type A to inactivation by certain detergents (Oakite, No. 63, and Ivory soap) (27).

The foregoing summary of literature dealing with the use of various agents and compounds for the recovery and isolation of certain viruses and other organisms from admixtures with various bacteria indicates generally a superiority of certain antibiotics, especially streptomycin and penicillin, over various sulfa and other compounds. Streptomycin appears to have a relatively wide range of usage and effectiveness, which often may be augmented by combination with penicillin. Certain demonstrated inadequa-

in studies involving antibacterial agents, little has been reported on the effect of these substances on bacterially contaminated egg tissues. Accidentally contaminated influenza-infected allantoic fluids were freed of the contaminating bacteria by contact with 1:10,000 zephiran at room temperature for 20 minutes (28).

The results of Morgan and Wiseman (36) indicated that 25 mg per cent sodium sulfadiazine and 125 units/ml of streptomycin can be added to 10 per cent yolk sac seed cultures of strain 6 BC psittacosis virus as a safeguard against possible contamination. Preliminary experiments of these investigators indicated the value of the bacteriostatic agents in dilution fluids (50 mg per cent sulfadiazine and 250 units/ml streptomycin) in the isolation of psittacosis virus by intracerebral injection in mice from such contaminated sources as *urine, feces, and soil.*

Other Considerations

Additional factors that may bear on the results of efforts to recover and isolate viral or other agents from environments harboring a variety of microbial flora have been given attention.

The effect of a certain concentration of the antibacterial substance or substances in any given material is obviously dependent on time, temperature, and other factors Interference with or protection against antibiotics by physical factors or mechanisms has also been recognized. The usefulness of an antibiotic palpably may be curtailed or limited because of rapid deterioration in or removal from the menstruum or host tissue.

Determinations of penicillin levels at intervals following injection into the yolk sac emphasized the fact that penicillin is destroyed rather rapidly from the fluids, there was approximately 0.3 units/ml 5 days after incubation as compared with 10 units/ml at initiation of the experiment (39). Introduction upon the serosa also resulted in a relatively rapid loss comparable to that which occurs in the tissues of various animals. Only 20 Oxford units of penicillin per milliliter were demonstrable in the embryonic fluids of chicken eggs at the 5th day after the beginning of five daily injections of 100 units per egg (43)

The deterioration of penicillin in tissue cultures was likewise demonstrated over a period of incubation. After an initial concentration of 10 units/ml, only 3.0 to 5 2 units were demonstrable in infected cultures 5 days later. After 17 days incubation in noninfected cultures, 0 2 unit per milliliter remained (39).

Relatively high stability or persistence of streptomycin following injection of the yolk sac of 7-day eggs was reported by Morgan *et al.* (35), who stated that this agent was not inactivated after 4 to 7 days. Assays of al-

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cies and limitations mediated by the host, the parasite, or the contaminant flora must, however, be recognized in contemplating and applying each antibacterial agent and method.

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No serious instances of toxicity were observed after parenteral or topical administration. Damage to the eighth cranial nerve was manifested in an 8-year-old Spitz-terrier cross being treated for cystitis. This animal had been treated unsuccessfully with several other antibiotics previously. The patient was then administered 10 mg of streptomycin per pound of body weight at 6-hour intervals for 14 days. On the 15th day, examination revealed the presence of vertigo, which could only be attributed to the drug. By the 5th day following cessation of the treatment, no symptoms were present.

In those cases in both cats and dogs where external otitis and conjunctivitis are caused by a predominance of gram-negative organisms, topical application solutions containing 25 to 50 mg of streptomycin per milliliter have been effective. To effect a satisfactory response solutions containing 100 mg/ml were necessary in cases where excessive exudate was present. Applications were made twice daily and continued until healing occurred.

Streptomycin is a very potent intestinal antibacterial agent because its absorption from the tract is very slow, if it takes place at all (2). Administered orally, it is useful in the treatment of those diarrheas in which an antibiotic is indicated. Diarrheas produced by the *Proteus* group of organisms usually responded satisfactorily, and many times symptoms ceased after the first oral administration. The dosage employed in such cases was 1 gm a day or in divided doses two or three times daily. The treatment was continued for 2 or 3 days, after which the stool usually appeared normal. In cases of acute diarrhea with much vomiting, the drug is not retained long enough to be of therapeutic value. A few of these cases were treated, with some success, with intraperitoneal and rectal injections.

Craige (3) reported on the use of streptomycin in the treatment of 139 dogs with dysentery. The mode of administration was found to be important in obtaining maximum results. In preparing the drug for oral administration, 1 gm was dissolved in 20 ml of sterile distilled water. The size of the dog and the severity of the symptoms directed the dosage employed. A dose of 1 to 10 ml (50 to 500 mg) was administered perorally. The results were much more satisfactory in the dysenteries associated with the *Proteus* group than in those of other etiology (table 82).

In dysenteries caused by coccidia, giardia, spirochetes, or in undiagnosed cases, Craige found streptomycin was not effective. Dysentery caused by spirochetal organisms usually responds more satisfactorily to a combination of sulfathalidine, penicillin, and streptomycin. The true role of streptomycin, in combination with other drugs, is difficult to evaluate.

Oral administration of streptomycin has been found to be very effective in controlling diarrheas in dogs infected with secondary invaders following the virus of Carré. For treatment of such cases, a single one-third gm dose

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CHAPTER 13

STREPTOMYCIN IN SMALL ANIMAL MEDICINE

A number of disease conditions in small animals may be produced by organisms that have been demonstrated to be susceptible to streptomycin. The more common of these conditions are external otitis, conjunctivitis, enteritis, pneumonia, suppuration of the anal glands, nephritis, cystitis, vaginitis, and tularemia. At present the limited use of streptomycin for such conditions in dogs and cats will not permit an accurate evaluation of its efficiency

CLINICAL TREATMENTS

In the absence of extensive information on the parenteral administration of streptomycin in dogs, the dosage used in the treatment of clinical cases at The Ohio State University Veterinary Clinic was based on estimations of the concentration in the blood serum and urine as determined by Smith (1) It was found that a dose of 5 mg per pound of body weight results in a blood serum concentration of approximately 23 units ml at the end of the first hour. This falls to approximately 6 units ml at the end of 4 hours A dose of 10 mg per pound of body weight results in a concentration of approximately 53 units ml at the end of the first hour This falls to approximately 6 units at the end of 6 hours With the 10-mg dose the interval may be increased to 6 hours, when necessary (for example during the night) and still maintain a terminal blood level no lower than that which results with the smaller dose at 4-hour intervals

Smith further observed that at 9 hours after a single intramuscular injection of 5 mg per pound of body weight, the urine concentration was above 100 units/ml The urine concentrations were found to be much more variable than those of the blood serum

Each case was treated as an individual problem Sensitivity tests were conducted whenever practical to direct the manner of dosing Where blood levels were of major importance, streptomycin was used in amounts of not less than 5 mg per pound of body weight at 4-hour intervals When the severity of the condition demanded a greater concentration, dosage of 10 mg per pound of body weight was used.

Streptomycin, penicillin, or combined streptomycin-penicillin treatment of dog patients with nervous symptoms following the virus of Carré has not been successful, in the experience of the authors. Table 84 illustrates the usual results in attempting to treat such dogs. The fever is maintained,

TABLE 83

Response of dogs with secondary infections following canine distemper virus to treatment with streptomycin orally and penicillin (200,000 units once daily, parenterally for 3 consecutive days)

CASE NUMBER	DAY OF TREATMENT	TEMPERATURE	STREPTOMYCIN ORALLY	CONGESTED MUCOUS MEMBRANES	NERVOUS SYMPTOMS	COUGH	NASAL DISCHARGE	DIARRHEA
		°F	gm					
1727	1st	103.2	0.33	+	−	+	+	+
	2nd	102.8	0.33	+	−	+	+	+
	3rd	101.8	0.33	+	−	−	+	−
	4th	101.6	—	−	−	−	+	−
	5th	101.8	—	−	−	−	−	−
	6th	101.8	—	−	−	−	−	−
						−	−	
1572	1st	104.0	0 50	+	−	+	+	+
	2nd	103.2	0.50	+	−	+	+	+
	3rd	103.2	0 50	+	−	+	+	+
	4th	102.0	0 33	+	−	+	+	−
	5th	101 8	—	+	−	−	+	−
	6th	101 8	—	−	−	+	−	−
	7th	101.6	—	−	−	−	−	−
							−	
1422	1st	104.8	0 33	+	−	+	+	+
	2nd	103 0	0 33	+	−	+	+	+
	3rd	103.4	0.33	+	−	−	+	+
	4th	102.8	0 33	+	−	−	+	−
	5th	102 0	—	+	−	−	+	−
	6th	102 2	—	+	−	−	+	−
	7th	101.8	—	−	−	+	−	−
	8th	101.4	—	−	−	−	−	−
							−	

the nervous symptoms become progressively worse, the cough, if present, and the nasal discharge continue throughout the course of the disease. On the other hand, Kellberg (5) is of the opinion that streptomycin may prove to be helpful in the treatment of such patients. The number of such cases treated with streptomycin have been too few to justify conclusions.

daily for at least 3 days was administered to 5- to 8-pound puppies. For dogs weighing 20 to 35 pounds, a single 1-gm dose daily was administered for at least 3 days. If the diarrhea was not controlled by the third day of treatment, the patient usually did not respond to further treatment.

Parenteral administration has been employed in the treatment of secondary gram-negative bacillary infections complicating canine distemper. The dosage of streptomycin recommended is 0.25 gm daily in divided doses of 30 to 40 mg subcutaneously for a 20- to 30-pound dog (4).

Pneumonias in dogs following the virus of Carré caused by secondary invasion of *B. bronchisepticus* and other organisms appear to be best treated with a combination of penicillin and streptomycin. Twenty such cases have been treated, with 80 per cent recovery. The treatment consisted of the daily intramuscular injection of 200,000 units of penicillin sodium crystalline G and 500,000 units of streptomycin for 3 or 4 days. If the patients did not show marked improvement after the third and fourth day they

TABLE 82

Effect of streptomycin treatment on dysentery in dogs (3)

	ACUTE BACILLARY DYSENTERY (*Proteus* GROUP)	CHRONIC BACILLARY DYSENTERY (*Proteus* GROUP)	OTHER INTESTINAL INFECTIONS	UNDIAGNOSED DYSENTERIES	TOTAL	PERCENTAGE
Immediate good results	53	2	2	3	60	43 2
Good results after prolonged treatment	16	17	18	6	57	41 0
Unsatisfactory results	0	5	13	4	22	15 8
Totals	69	24	33	13	139	100.0

rarely responded to further treatment. These cases were not complicated with severe diarrhea and nervous symptoms. In the treatment of fifty such cases with penicillin alone (200,000 units daily in a single intramuscular dose for 3 days) 76 per cent recovered.

In cases where secondary invasion following the virus of Carré involves both the digestive tract and the pulmonary system, the most successful treatment appears to be a daily oral dose of one-third to 1 gm of streptomycin together with a daily intramuscular injection of 200,000 units of penicillin This treatment is continued for 3 to 4 days Table 83 illustrates the usual response in cases that recover The fever decline is rapid, and the diarrhea ceases within 3 to 4 days. If such a response is not effected within 3 or 4 days, further treatment with penicillin and streptomycin is without result.

of 100 units of streptomycin per milliliter, a therapeutically beneficial concentration, will be maintained following such a dosage. Cystitis cases caused by gram-negative organisms (coli group) usually show marked improvement after treatment for 3 to 7 days. Kellberg (5) has reported similar observations on several cystitis cases. One case of acute nephritis in the dog was successfully treated with streptomycin (6).

In cases of cystitis where the underlying etiology is a malignant neoplasm, permanent recovery after treatment with streptomycin does not result. Streptomycin in these cases does, however, improve the inflammatory condition resulting from secondary invasion of organisms. Treatment must be repeated at varying intervals until the neoplasm is removed surgically.

One patient with a possible *P. tularensis* infection was treated with streptomycin. The patient had an agglutination titer for *P. tularensis* in the dilution of complete at 1:80. Prior to treatment with streptomycin, all other antibiotics had failed to produce a response. After treatment with a daily intramuscular injection of 1 gm of streptomycin for 4 consecutive days, the patient responded rapidly and recovered completely.

One case of "mouth rot" in snakes was treated. Previous to treatment, a species of *Pseudomonas* was isolated and tested for sensitivity. The snake was given 10 mg per pound of body weight at 6-hour intervals for 7 days. Recovery was complete. So far as we know, this disease is otherwise fatal.

Comment

From the authors' experience with streptomycin in the treatment of clinical cases in small animals, the drug is most useful in treating infections caused by gram-negative organisms. It may also be of value in treating certain infections caused by penicillin-resistant, streptomycin-sensitive, gram-positive organisms. More research will be necessary to confirm this observation. As is true of all antibacterial agents, streptomycin should be considered not as a substitute for surgery or other established therapeutic methods but only as an adjunct to treatment in those cases where the drug is indicated.

The optimum dosages appear to vary with the streptomycin-sensitivity of the pathogen and with the type, stage, severity, and location of the infection. The wide variation in susceptibility to streptomycin among different bacterial species has been well shown by Coles (7). The variation in susceptibility among different strains of the same bacterial species is also important For example, Coles found among the strains of *S. aureus* studied, that those isolated from dogs showed more susceptibility to the action of streptomycin than did the staphylococci isolated from other sources.

In the author's experience, intramuscular administration of streptomycin in the treatment of systemic infections is the method of choice. Subcu-

Kellberg (5) reported some success in the treatment of feline distemper with daily administration, subcutaneously, of 80 to 120 mg streptomycin in divided doses of 10 to 15 mg.

TABLE 84

Response of dogs with secondary infections following canine distemper virus to treatment with streptomycin subcutaneously and penicillin (200,000 units once daily, subcutaneously)

CASE NUMBER	DAY OF TREATMENT	TEMPERATURE (for 3 consecutive days)	STREPTOMYCIN SUBCUTANEOUSLY	CONGESTED MUCOUS MEMBER	NERVOUS SYMPTOMS	COUGH	NASAL DISCHARGE	DIARRHEA
		°F	gm.					
2078	1st	105 0	0 50	+	−	−	+	−
	2nd	103.0	0 50	+	−	−	+	−
	3rd	102 6	0 50	+	+	−	+	−
	4th	102 8		+	+	−	+	−
	5th	103 2	−	+	+	−	+	−
	6th	103 0	−	+	+	−	+	+
	7th	103 0	−	+	+	−	+	+
	8th	102 6		+	+	−	+	+
	9th	Euthanasia						
1787	1st	102 6	1 00	+	+	+	+	−
	2nd	105 6	1 00	+	+	+	+	−
	3rd	105 3	1 00	+	+	+	+	−
	4th	104 0	−	+	+	+	+	−
	5th	104 8	−	+	+	+	+	−
	6th	103 8	−	+	+	+	+	−
	7th	106 0	−	+	+	+	+	−
	8th	106 0		+	+	+	+	−
	9th	Euthanasia						
1387	1st	104 6	50	+	+	−	+	−
	2nd	104 0	50	+	+	−	+	−
	3rd	103 6	50	+	+	−	+	−
	4th	103 8	−	+	+	−	+	−
	5th	103 6	−	+	+	−	+	−
	6th	103 6	−	+	+	−	+	−
	7th	105 0	−	+	+	−	+	−
	8th	Patient died						

Urogenital infections caused by gram-negative organisms (coli group) in dogs usually respond well to treatment with streptomycin. Excellent results have been obtained in the treatment of six cases of cystitis by employing 5 mg per pound of body weight and injecting the drug intramuscularly every 8 hours. Smith's (1) results indicate that a urine concentration

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CHAPTER 11

MYCIN IN THE TREATMENT OF S INFECTIONS OF THE BOVINE MAMMARY GLAND

and Brown (1) studied streptomycin in relation to the gland in five grade Holstein cattle and one grade Toggen- summarized their trials as follows: 1. Streptomycin in- mal bovine mammary gland in amounts ranging from units per quarter could be detected in milk samples as following infusion. As determined by the assay pro- concentration did not fall below 20 units ml in any of the 24-hour interval. 2. The concentration per milliliter in and to vary with (a) the size of the dose, (b) time interval and sampling, and (c) milk production of individual to time was there sufficient streptomycin present in the cted by the assay procedure used. However, in both the ificant amounts were found in urine samples as long as ng infusion. 4. Under the conditions of these experi- ycin was found to be relatively nontoxic when infused into he mammary gland.

successfully treated one bovine mammary quarter, affected astitis caused by *E. coli*, by infusing 500,000 units of strepto- ntervals, 12 hours apart. Lipmann (3) treated three cases es caused by *E. coli* infection. In the initial case, this ob- d 0.5 gm of streptomycin twice at 12-hour intervals, followed d 0.25 gm of streptomycin once daily for 4 days. The second ed by infusing 0.25 gm of streptomycin four times at daily a total of 1.0 gm of streptomycin . In all of three cases com- recovery occurred by *E. coli*. The animal had not re- (4) used streptomycin

Drew, senior laboratory techni- invaluable assistance.

Kellberg (5) reported some success in the treatment of f with daily administration, subcutaneously, of 80 to 120 i in divided doses of 10 to 15 mg.

TABLE 84

Response of dogs with secondary infections following can treatment with streptomycin subcutaneously a (200,000 units once daily, subcutane

CASE NUMBER	DAY OF TREATMENT	TEMPERATURE (for 3 consecutive days)	STREPTOMYCIN SUBCUTANEOUSLY (for 3 consecutive days)	CONGESTED MUCOUS MEMBER	NERVOUS SYM
		°F	cm.		
2078	1st	105.0	0.50	+	
	2nd	103 0	0 50	+	
	3rd	102 6	0 50	+	
	4th	102.8	–	+	
	5th	103 2	–	+	
	6th	103 0	–		
	7th	103 0	–		
	8th	102 6	–		
	9th	Euthanasia			
1787	1st	102.6	1.00		
	2nd	105.6	1.00		
	3rd	105.3	1.00		
	4th	104.0	—		
	5th	104 8	—		
	6th	103.8	–		
	7th	106.0	–		
	8th	106.0			
	9th	Euthanasi			
1387	1st	104.6			
	2nd	104.0			
	3rd	103.6			
	4th	.8			
	5th	.6			

sponded to sulfamethazine orally or to penicillin administered both by infusion and by intramuscular injection. The goat recovered following the infusion of 1 gm of streptomycin divided into five equal doses. These reports, though limited in number, point to the usefulness of streptomycin in the treatment of clinical mastitis caused by infection with *E. coli*.

In our investigations (5), a preliminary report of which has been published, mammary glands infected with a variety of organisms were treated. In addition to *E. coli* these included *A. aerogenes*, *A. cloacae*, *Ps. aeruginosa*, and *S. aureus*. The data summarized in this chapter were collected in a single large dairy herd. The streptomycin employed initially, consisted of streptomycin base in the form of the trihydrochloride, but the major portion of the streptomycin used in our trials was the calcium chloride complex.[2]

Methods

Herd management

The dairy herd in which these studies were made is owned and operated by one of the California State Hospitals for mental patients. It is composed of both grade and purebred Holstein cows which are assigned to milking strings consisting of not more than 30 animals each. The string to which a cow is assigned is determined by the nature of the bacterial flora of her udder, and the cows and the strings are milked in a sequence designed to retard the spread of mammary gland infections. Between milkings each string is kept in a separate paved corral having an adjoining shelter shed which is kept thickly bedded with clean straw or wood shavings. About 150 of the cows are milked at 12-hour intervals and about sixty additional cows are milked at 8-hour intervals. The milking is done by machines operated by hired employees. Selected inmates of the institution follow the machines and hand-strip the cows. The teats are then dipped in a solution containing between 250 and 400 p.p.m. of available chlorine.

History of the mastitis project

A mastitis control program was initiated in this herd in May 1943. At first, emphasis was placed on the control of chronic mastitis caused by *S. agalactiae*, hence, the program was based on routine bacteriologic analysis of milk samples, segregation, and therapy. By the fall of 1945 this pathogen had been almost entirely eliminated. The project was continued with major effort directed toward a reduction in incidence of mammary gland infections caused by *S. aureus*. Early in 1947, however, it became apparent that mastitis caused by organisms of the coliform group was occurring in a sporadic fashion. Soon thereafter, both acute and chronic

[2] Streptomycin base was provided by Merck and Company, Inc., Rahway, New Jersey.

mastitis due to coliform organisms appeared at such an alarming rate that it became necessary to shift the major effort of investigational work to a study of the coliform infections.

Bacteriologic procedures

Composite milk samples were drawn at weekly intervals from cows in selected segments of the herd. In this manner the entire lactating herd was sampled for bacteriologic study at least once every 3 months, whereas animals showing clinical mastitis or cows that had received specific treatment for mammary gland infections, were sampled at weekly intervals as long as necessary to complete the data.

The milk (15–18 ml) was collected directly into screw-cap vials containing 1.0 ml of a 0.33 per cent aqueous bromcresol purple solution (Hotis test). Milk samples were incubated at 37°C for 16 to 20 hours. A loopful of each incubated milk sample was streaked on half of the surface of a veal infusion agar plate containing fresh cow blood. Single colonies of bacteria were transferred for pure culture studies to beef extract broth containing a small quantity of bovine serum.

Specific identification of the coliform organisms was made by the "IMVIC" method of Parr (6) supplemented by observations on acid and gas production by the cultures in nutrient broth containing 1.0 per cent lactose, sucrose, or glycerol.

Coliform Mastitis

Infection statistics

During the course of 1 year, and prior to the use of streptomycin in the herd, 270 cows were observed. A cow was regarded as having a coliform infection in her udder only when the specific organism was demonstrated in the same mammary quarter on two or more samplings a week or more apart. Failure to repeat, after first isolation, occurred frequently with each of the coliform types identified. Infections, according to this criterion, were found in a total of ninety-six quarters and were distributed as follows: *A. aerogenes*, fifty-one quarters, *A. cloacae*, sixteen quarters; *E. coli*, twenty-seven quarters; and unclassified coliforms, two quarters. In addition, a few infections with intermediate coliform types were encountered, but they did not present a serious clinical problem.

Clinical manifestations

The coliform infections were classified on the basis of clinical manifestations as latent, chronic, acute local, or acute systemic. A brief description of each follows:

LATENT

Infections described as latent usually persisted for periods ranging from several weeks to 8 months, and they were not accompanied by a detectable alteration in the milk. About 40 per cent of the infections were classified as *latent.*

CHRONIC

Infections were classified as chronic when accompanied by evidence of more or less mild tissue irritation. This form of the disease was characterized by the intermittent occurrence of visible particles in the first milk, periodic increases in leucocyte number in the milk, fluctuations in pH of the milk, and intermittent transitory swellings of the quarter. Chronic infections usually persisted for months. For example, one quarter remained infected with *A. cloacae* for 17 months, and another quarter shed *A. aerogenes* for 22 months. Both of these infections were later removed by streptomycin infusions. One case of chronic mastitis of 2 months' duration terminated in acute systemic mastitis.

ACUTE LOCAL

The acute local form of coliform mastitis usually occurred without previous indication of infection in the quarter, although in some instances a recent latent infection terminated in acute local mastitis. The first stage usually consisted of the sudden appearance of a soft edematous swelling of the parenchyma immediately above the gland cistern. The quarter was often warm and sensitive and the secretion, though milk-like, usually contained small to very large mucofibrinous clots. In some cases the symptoms would begin to subside by the next milking period, and in a few days the quarter would return to normal. In the majority of cases, the entire quarter would exhibit a more or less soft edematous swelling, or the condition would have progressed to an intense firmness throughout the parenchyma by the next milking period. The termination of such cases was variable. Either the swelling receded and the quarter returned to the production of normal milk within the next few days, or the regression of the swelling occurred more slowly, leaving a partly atrophied quarter. To what extent these partial or complete recoveries were influenced by supportive treatment was not determined. Acute swelling was invariably treated by soaking the udder in hot water twice daily for 30-minute intervals. In addition, the involved quarter was milked-out completely and then massaged thoroughly. In some cases intramammary therapy was used in addition. This included infusions of penicillin in repeated 100,000-unit doses and/or sulfamethazine either as a diluent for the penicillin or given systemically. The response to such treatment was exceedingly variable and, hence unsatisfactory

ACUTE SYSTEMIC

The reaction of the involved mammary quarter in acute systemic mastitis was similar to that in acute local mastitis, but in addition a systemic reaction occurred. The latter consisted of inappetence, partial to complete cessation of milk flow, an abnormal flow of mucoserous fluid from the nostrils, fluctuations in rectal temperatures between 102° and 108.3°F, and rapid loss of weight. In the more severe cases an edematous swelling of the hock and pastern joints occurred, and in one case an extensive edema developed in the ventral abdominal wall between the anterior border of the udder and the sternum. In the most extreme forms of the disease, the cows died in 5 to 30 days. The acute systemic form usually occurred early in lactation. Such cows usually had not yet rebred, had ceased to lactate, and had lost considerable flesh. Hence, they were unprofitable for dairy purposes and had to be culled from the herd. Prior to the use of streptomycin in this herd, fourteen cows developed acute systemic mastitis. *A. aerogenes* was associated with seven of these cases, *E. coli* with five cases, and in two cases the coliform type was not identified. Three of these cows died, ten became culls, and one recovered sufficiently to be milked again.

3. Streptomycin Studies

Technic

The streptomycin was prepared for infusion by dissolving it in sterile, distilled water in the ratio of 1 gm of streptomycin base to 100 ml of water. The udder was washed free of extraneous organic material before treatment. The teat orifice was disinfected with alcohol immediately before the streptomycin infusion was administered. A sterile ground-glass syringe fitted with a sterile metal cannula was used to introduce the infusion into the quarter. Thereafter, the cisterns of the teat and gland were massaged briefly. Soaking the udder in hot water for 30 minutes after the night and morning milkings was continued in all cases of acute local and acute systemic mastitis.

Dosage

The total quantity of streptomycin infused into an individual mammary quarter in a single course of treatments was varied between 1.5 and 8.5 gm. The quantity employed for a single dose within the series was either 0.5 or 1.0 gm with the exception of two quarters which received initial doses of 2.0 and 2.5 gm, respectively. A total of 1.5 or 2.0 gm of streptomycin did not yield satisfactory results in lactating cows having chronic, acute local, or acute systemic mastitis caused by *A. aerogenes*. Subsequent observations on these animals suggested the possibility that serious consequences might ensue from use of an insufficient quantity of streptomycin. Specific instances in which this appeared to be the case were as follows:

1. Cow 1342 developed acute local mastitis in her right rear quarter. Infusions of 0.5 gm of streptomycin were administered four times at 12-hour intervals (total 2.0 gm). The acute symptoms subsided but the infection remained and became chronic. One month later the same quarter was given eight 0.5-gm infusions of streptomycin at 12-hour intervals. Still the infection with *A. aerogenes* was not removed and symptoms of chronic mastitis continued to appear intermittently. Eight weeks after the second series of infusions, a third attempt was made to remove the infection. This time 1.0 gm of streptomycin was administered every 12 hours until a total of 8 gm had been infused into the quarter. Following treatment with this large total dose of streptomycin, the *A. aerogenes* infection disappeared and the quarter returned to the secretion of normal milk.

2. Cow 1457, infected with *A. aerogenes* in her right rear quarter, had shown chronic mastitis for 5 months. The involved quarter was infused with a total of 2.0 gm of streptomycin, administered in 0.5-gm doses at 24-hour intervals. The infection persisted, and 2 months later the quarter was retreated with a total of 4.0 gm of streptomycin. Subsequent samplings of the quarter revealed that *A. aerogenes* was still present. A third treatment was given, consisting of a total of 8 gm of streptomycin, administered in 1.0-gm doses every 12 hours for 4 days. In this cow, the third series of infusions with a large total dose of streptomycin failed to remove *A. aerogenes*. No trials were made to ascertain whether this strain of *A. aerogenes* had become resistant to streptomycin, but it was suspected that such might have been the case. Thereafter, the cow was milked last to preclude the spread of this possibly resistant strain to other cows in the herd

3. Two lactating cows, 1685 and 1713, developed acute systemic mastitis caused by *A aerogenes*. The involved quarter in each cow received a total of 1 5 gm of streptomycin, administered in 0.5-gm doses at 12-hour intervals. In cow 1685, the symptoms subsided but the infection persisted in the involved quarter for 1 week after treatment and then disappeared. Similarly, in cow 1713 the symptoms began to subside; however, a relapse occurred 72 hours after the last infusion of streptomycin. Rapid deterioration to a cull condition followed despite an additional treatment with 3.5 gm of streptomycin which was infused into the involved quarter during the succeeding 3 days This experience raises the question of whether the relapse in cow 1713 could have been prevented had the initial treatment been extended for several more days

4. Cow 1375 was a valuable animal, having produced 881 pounds of fat in 330 days on a three-time milking schedule. She showed a latent infection with *A. aerogenes* in her left front quarter. Streptomycin was infused

in 0.5-gm doses at noon and at 8 p.m. on two successive days (total 2.0 gm). Two weeks after treatment, a milk sample from this quarter contained *E. coli*, which could have been a contaminant. Ten days subsequent to that observation, the left front quarter developed acute local mastitis. The herdsman immediately instituted treatment with streptomycin, but he overlooked drawing a pretreatment milk sample for bacteriologic study. This second series of streptomycin infusions was identical in scope with the first series and, therefore, a total of 2.0 gm was again administered. The symptoms subsided and the quarter returned to secreting a normal milk. During the next 7.5 months, milk samples were drawn for bacteriologic study on twelve occasions No coliform organisms were found in the first eight samplings taken during the first 6 months. *A. aerogenes* was isolated from both the left front and right rear quarters at the 9th, 11th, and 12th samplings, but not at the 10th sampling. This represented a recurrence of *A. aerogenes* in the left front quarter and a new infection in the right rear one. At the time the 12th sample was drawn, the cow was dry. A decision was made to treat the two infected quarters. Since the left front quarter had been treated on two previous occasions with 2.0-gm doses of streptomycin, it was decided to increase the dose in the current treatment. The left front quarter was treated with 8 gm of streptomycin, whereas the right rear quarter was treated with 4 gm. These quantities were given in four equal doses at 24-hour intervals. The cow calved 7 days after the infusions were completed. Milk samples drawn twice during the first week after calving were negative for coliform organisms, but 6 weeks later the cow developed acute systemic mastitis in her right rear quarter. The day prior to the occurrence of the acute mastitis, this cow produced a total of 81.8 pounds of milk. At the 4 a.m. milking on the day of appearance of the mastitis, her udder was normal and produced at this milking 27.5 pounds of milk, whereas 8 hours later, when she was brought in for the second of her three daily milkings, her right rear quarter was swollen and the total production from her udder was only 8.0 pounds of milk. At 8 that night, she walked with difficulty, her nostrils were discharging a mucoserous material, her rectal temperature was 107.2°F, and her total milk production was only 5.2 pounds. Pretreatment milk samples were drawn, and *A. aerogenes* was found in the sample from the right rear quarter. Because of her previous history relative to streptomycin treatments, a large initial dose, consisting of 2.5 gm of streptomycin, was infused Subsequent treatments and observations were as follows:

2nd day At 4 a.m., temp 106.2°F. Infused 1.0 gm of streptomycin and 100,000 units of penicillin Also, 0.5 gm of streptomycin administered subcutaneously. Temp at 9 p.m. 101.2°F

3rd day: At 4 a.m., temp. 103.4°F. Infused 0.5 gm of streptomycin and gave an additional 0.5 gm subcutaneously. Noon, temp. 103.6°F. Considerable improvement in general appearance.

4th day: At 8 a.m., cow is eating hay, and temp. is 103.4°F. Infused 0.5 gm of streptomycin. At 9 p.m., temp. 104.6°F.

5th day: At 9 a.m., cow is not eating. She appears very sick. Infused 0.5 gm streptomycin at 9 a.m. and again at 9 p.m. Also gave 0.5 gm of streptomycin subcutaneously at 9 a.m. Temp. at 9 p.m. 104.7°F.

6th day: At 7 a.m., cow is very sick, is off-feed, and has forced respiration. Temp. 105.8°F. Right rear quarter markedly swollen. Gave 1.0 gm of streptomycin subcutaneously at noon and again at 8 p.m. Temp at 9 p.m., 105.1°F.

7th day: At 7 a.m., temp. 105.1°F. No milk from udder. Cow still very sick. Has forced respiration. Gave 4.0 gm of streptomycin subcutaneously, followed at noon by an infusion of 1.0 gm of streptomycin into the involved quarter and administration of 1.0 gm subcutaneously. In addition, 450 grains of sulfamethazine was given by mouth. At 9 p.m., 1.0 gm of streptomycin infused and 1.0 gm given subcutaneously.

8th day: At 6 a.m., temp. 103.6°F. Cow is eating hay. Gave 1.0 gm of streptomycin by infusion and 1.0 gm subcutaneously. Right rear quarter still firm but softening. At 4 p.m., temp. 105.4°F. Gave 570 grains of sulfamethazine by mouth.

9th to 20th day: Gradual return to normal appetite and body temperature. Loss of weight during illness not excessive. Cow completely dry and nonpregnant, but her value as a breeding animal justifies retaining her in the herd.

In speculating on the case of cow 1375, it appears possible that a strain of *A. aerogenes*, which had become relatively resistant to streptomycin, had developed following the two previous treatments of the left front quarter with 2.0 gm of the antibiotic. This may have resulted in a latent infection in that quarter, from which the organism was shed intermittently. If such were the case, it is possible that the right rear quarter became infected as a result of spread of *A. aerogenes* from the left front quarter. The infection in the left front quarter yielded to treatment with a total of 8 gm of streptomycin administered during the dry period, whereas the infection in the right rear quarter was treated with a total of only 4 gm, and apparently *A. aerogenes* was not removed from that quarter, and later an acute systemic mastitis developed. It is equally possible that the acute systemic mastitis originating in the right rear quarter was a recent, new infection with a streptomycin-resistant strain.

5. The case of cow 1642 strongly suggests that streptomycin-resistant strains of *A. aerogenes had appeared in the environment of the herd.* This cow developed acute mastitis of the left front quarter during the third month of lactation. *A. aerogenes* was isolated from a pretreatment sample. Twelve hours after symptoms were first observed, an infusion of 2.0 gm of streptomycin was administered. This was followed by additional

infusions, given at five 12-hour intervals and consisting of 1.0 gm, 1.0 gm, 1.0 gm plus 400,000 units of penicillin, 0.5 gm, and 1.0 gm, respectively (total 6.5 gm). At the time of the initial infusion of 2.0 gm, the cow's temperature was 104.6°F. During the remainder of the treatment period her temperature fluctuated between 101.8° and 103.7°F. The cow showed inappetence, but she was eating again on the fourth day. The involved quarter became very firm, and an edematous swelling extended into the left flank. The swelling of the parenchyma did not soften during or after

TABLE 85

Results obtained when lactating quarters, infected with various pathogens, were treated with 4.0 gm of streptomycin, administered in 0.5-gm doses, twice daily for 4 days

PATHOGEN	CLINICAL CLASSIFICATION	NUMBER OF QUARTERS		REMARKS
		Treated	Cured	
A. aerogenes	Latent	2	2	—
	Chronic	7	7	—
	Acute local	2	1	1 qtr. symptoms subsided but infection remained 2 months
	Acute systemic	3	1	1 cow died. 1 cow became a cull.
A. cloacae	Latent	4	4	—
	Chronic	5	3	Infections persisted in 2 qtrs
	Acute local	1	1	—
E. coli	Chronic	1	1	—
	Acute local	3	3	—
	Acute systemic	4	3	1 cow became a cull
Ps. aeruginosa	Latent	2	0	—
	Chronic	4	0	—
S. aureus	Latent	1	0	—
	Chronic	1	0	—

treatment, and the quarter gave only a small quantity of blood-tinged exudate. *A. aerogenes* was present in a sample drawn from the affected quarter 20 hours after the last infusion of streptomycin and was still present in another sample drawn 6 days later. The cow returned to a satisfactory milk flow from the three noninfected quarters, but the left front quarter remained firm. The animal was slaughtered as a precautionary measure to preclude spread of this possibly streptomycin-resistant strain to other cows in the herd.

Experience with these six cows suggests that a large total dose of streptomycin should be employed when coliform infections of the mammary gland are to be treated. It was decided, therefore, to try in lactating cows a total of 1.0 gm of streptomycin base per mammary quarter, and this was to be administered in 0.5-gm doses, twice daily, for 4 days. The following results were obtained with this pattern of treatment.

Results

Table 85 presents a summary of the results obtained from the administration of a total of 4 gm of streptomycin to lactating quarters infected with

TABLE 86

Results obtained when dry quarters, infected with various pathogens, were treated with either 2.0 or 4.0 gm of streptomycin, administered in 0.5- or 1.0-gm doses, respectively, once daily for 4 days

PATHOGEN	CLINICAL CLASSIFICATION	TOTAL STREPTOMYCIN	NUMBER OF QUARTERS		REMARKS
			Treated	Cured	
		gm			
A. aerogenes	Latent	2	3	3	—
	Chronic	4	1	1	—
E. coli	Latent	4	1	0	Acute local mastitis developed later. Cured with 8 gm
	Chronic	2	1	1	
Ps. aeruginosa	Chronic	4	1	0	Re-treated with 4 gm when lactating. Not cured
S. aureus	Latent	2	3	1	1 qtr. re-treated with 4 gm. Not cured
	Chronic	2	1	1	
	Chronic	4	1	1	

various bacterial pathogens. Of thirty-two quarters infected with *A. aerogenes*, *A. cloacae*, or *E. coli*, twenty-six responded satisfactorily. The six infections that did not respond satisfactorily were distributed as to type of infection and clinical classification as follows: *A. cloacae*, two quarters with chronic mastitis, *A. aerogenes*, one quarter with acute local mastitis and two quarters with acute systemic mastitis, and *E. coli*, one quarter with acute systemic mastitis. Of the three acute systemic infections which

terminated unsatisfactorily, one cow died and two cows became culls and were slaughtered. Streptomycin was also employed on four lactating quarters infected with *Ps. aeruginosa* and two lactating quarters infected with *S. aureus*. None of these infections were removed by treatment.

Table 86 presents a summary of streptomycin treatments administered to infected dry quarters. A total of either 2.0 or 4.0 gm of streptomycin, was given in 0.5- or 1.0-gm doses, respectively, once daily, for 4 days. Favorable responses were obtained in four quarters infected with *A. aerogenes*, in one of two quarters infected with *E. coli*, in three of five quarters infected with *S. aureus*, whereas one quarter infected with *Ps. aeruginosa* remained infected. The number of quarters treated is too limited to permit a conclusive statement relative to the total dose of streptomycin that should be used for best results in the treatment of infections in dry quarters.

Discussion and Summary

A. aerogenes, *A. cloacae*, or *E. coli* infections of the mammary gland, which, on the basis of clinical manifestations, were classified as latent, chronic, or acute local mastitis, have for the most part responded satisfactorily to intramammary therapy with streptomycin when infused in 0.5-gm doses, twice daily, for 4 days. Clinical evidence is presented which suggests that use of smaller total doses of streptomycin may lead to development of streptomycin-resistant strains of coliform organisms, specifically *A. aerogenes*. Certain observations presented in this paper indicate that such an enhanced resistance to streptomycin on the part of the pathogen may result in subsequent occurrence of cases of clinical mastitis which require very large total doses of streptomycin to produce beneficial results or which may fail to respond at all to streptomycin therapy.

Three of 7 cows affected with acute systemic mastitis caused by coliform organisms were not benefitted by intramammary therapy with a total dose of 4 grams of streptomycin. To date, no cultural studies have been made on the blood of cows showing systemic reactions in coliform mastitis. It is quite possible that in such cows a bacteriemia occurs. If this is correct, a combination of systemic and intramammary therapy with streptomycin is indicated.

Multiple infusions of streptomycin totaling 4 grams per lactating quarter have failed to remove *Ps. aeruginosa* from 6 quarters or *S. aureus* from two quarters. Further trials are indicated.

Dry quarters, infected with a variety of bacterial pathogens, have been given a total of either 2.0 or 4.0 grams of streptomycin, administered daily in 0.5- or 1.0-gram doses, respectively, for 4 days. Favorable re-

sponse was obtained with some of the cases but the data available at this time are too limited to be conclusive.

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CHAPTER 45

STREPTOMYCIN AND PENICILLIN-STREPTOMYCIN FOR CONTROL OF BACTERIA IN BOVINE SEMEN

One of the most important problems in artificial insemination of dairy cattle is control of the bacterial flora of diluted bull semen. Present-day techniques for the collection of bull semen tend to reduce bacterial contamination, but it still is impossible to obtain semen which is absolutely free of bacteria. Further contamination may occur prior to insemination of the female unless adequate precautions are taken in the preparation and handling of the diluted semen. In addition, both undiluted semen and diluted semen serve as excellent media for bacterial growth.

The presence of bacteria in semen may introduce error into the results of studies on the metabolism of spermatozoa. Bacteria compete with spermatozoa for nutrients and other substances. The toxins and end products of bacterial metabolism may be harmful to the viability of spermatozoa.

The possibility that the semen of the bull may serve as a means of transmitting certain genital infections which are related to fertility problems in females is of great significance in artificial insemination under field conditions. For example, it has been shown (1) that *Br. abortus* can be spread by artificial insemination with infected semen. Types of bacteria similar to those associated with metritis, cervicitis, sterility, and abortion in cows sometimes are found in the semen of bulls. Though no apparent relationship between numbers of bacteria in semen and fertility level of bulls has been found (2), certain types of bacteria, particularly *Ps. aeruginosa* (3) and members of the genus *Micrococcus* (4), appear to be related to low fertility in the bull.

On the basis of the foregoing facts, bacteriological control of bovine semen with antibacterial agents would appear to have practical value not only in metabolism studies but particularly in extending the usefulness of routine artificial insemination of dairy cattle.

In recent years various antibacterial agents, including streptomycin, penicillin, and various sulfonamides, have been used in efforts to control

bacterial growth in bull semen. In studies with penicillin, Almquist, Thorp, and Knodt (5) found that complete bacterial control could not be achieved with as high as 2,000 units/ml of diluted semen. Since penicillin-resistant organisms were encountered, Almquist *et al.* (6) next investigated the effectiveness of streptomycin.

Inhibition of Bacterial Growth by Streptomycin

In 1948, Almquist, Glantz, and Thorp (6) reported that streptomycin could be added to diluted bovine semen in amounts that would effectively retard the growth of bacteria without being harmful to the activity of the spermatozoa. Levels of streptomycin ranging from 100 to 2,000 μg or units/ml of diluted semen were studied. The semen was diluted at the constant rate of 1 part of fresh semen to 21 parts of egg yolk-citrate diluter. The yolk-citrate diluter was composed of 1 part of fresh egg yolk and 1 part of a buffer solution of 3.6 per cent sodium citrate dihydrate.

To be of practical value any antibiotic added to bull semen must not exert an injurious effect upon the spermatozoa. Relatively high concentrations of streptomycin can be added to diluted semen without apparent toxicity to the spermatozoa. Additions of 100, 250, 500, 750, and 1,000 units of streptomycin sulfate per milliliter of diluted semen did not significantly affect the viability of bull spermatozoa during storage for 20 days at 4.5°C. Levels of 1,250, 1,500, and 2,000 units/ml of diluted semen, however, brought about a significant decrease in the motility of the spermatozoa during storage.

Bacterial plate counts showed that streptomycin inhibited growth of bacteria in diluted semen as compared with semen containing no streptomycin. Levels above 100 units/ml of diluted semen were especially effective. Bacterial control was evident not only in freshly diluted semen, but also in diluted semen after storage for 8 days at 4.5°C. Thus the average plate count for the stored diluted semen containing no streptomycin was 82,000 bacteria per milliliter, whereas similar portions of semen containing 100 to 2,000 units of streptomycin per milliliter averaged only 2,000 bacteria per milliliter. In general, streptomycin appears to be more effective than penicillin (5) for controlling the growth of bacteria in diluted bull semen.

There was no significant loss in streptomycin activity in diluted semen stored for 8 and 16 days at 4.5°C, according to assays by the standard cylinder plate method using *B. subtilis* as the test organism. In the future, control of bacteria probably will become increasingly important as semen is used for insemination after undergoing longer periods of storage. Thus the marked stability of streptomycin is an important attribute so far as its practical use in diluted semen is concerned.

Since more than 100 units/ml of diluted semen are required for most efficient bacterial control, and since levels exceeding 1,000 units/ml are deleterious to the spermatozoa, it appears that the recommended concentrations for use in diluted bull semen are between 250 and 1,000 units/ml.

In studies to develop a synthetic diluting medium for bull semen, Phillips and Spitzer (7) noted considerable growth of bacteria in some of the preparations. They recommended that sulfathalidine, sulfasuxidine, or streptomycin be added to their synthetic pabulum (diluter) at the rate of 25 to 30 mg/100 ml for the control of bacterial growth. The streptomycin used by these workers assayed 190 units/mg; therefore, the pabulum contained 57 units/ml at the 30 mg per cent level (8). No data on the numbers of bacteria with and without this level of streptomycin were given. Though this level of streptomycin would be somewhat effective in controlling bacterial contamination higher dosages could be used safely, in light of findings described above, and would result in more efficient retardation of bacterial growth.

Penicillin-Streptomycin vs. Streptomycin for Bacteriological Control

Since neither penicillin nor streptomycin alone provided complete bacterial control in diluted bull semen, Almquist, Glantz, and Shaffer (9) studied the effect of a combination of the antibiotics. They found that a combination of penicillin and streptomycin definitely is more effective than either one alone. Also, there appeared to be a marked synergistic effect.

As shown in figure 90, eight combinations of the sodium salt of penicillin and streptomycin sulfate ranging from 100 to 1,000 units of each per milliliter of diluted bull semen were studied. Viability studies involving 1,170 motility estimations showed that none of the combinations caused a significant effect upon the ability of the spermatozoa to remain motile during a 20-day storage period. Apparently penicillin and streptomycin act independently in their effects upon spermatozoan viability, as the total effect when used together appears to be no greater than when either is used alone (5, 6).

In the first series of semen samples studied, Almquist, Glantz, and Shaffer (9) found no growth of bacteria on plates representing penicillin-streptomycin-treated semen either in freshly diluted semen or diluted semen stored for 8 days. Bacterial contamination of the undiluted semen samples used was very low, however, as shown by the average plate count of 5,700 per milliliter. To obtain higher, more representative bacterial counts in the untreated control semen, the freshly collected samples in the second series were inoculated with broth cultures of bacteria isolated from previous semen platings. The broth cultures consisted of five different

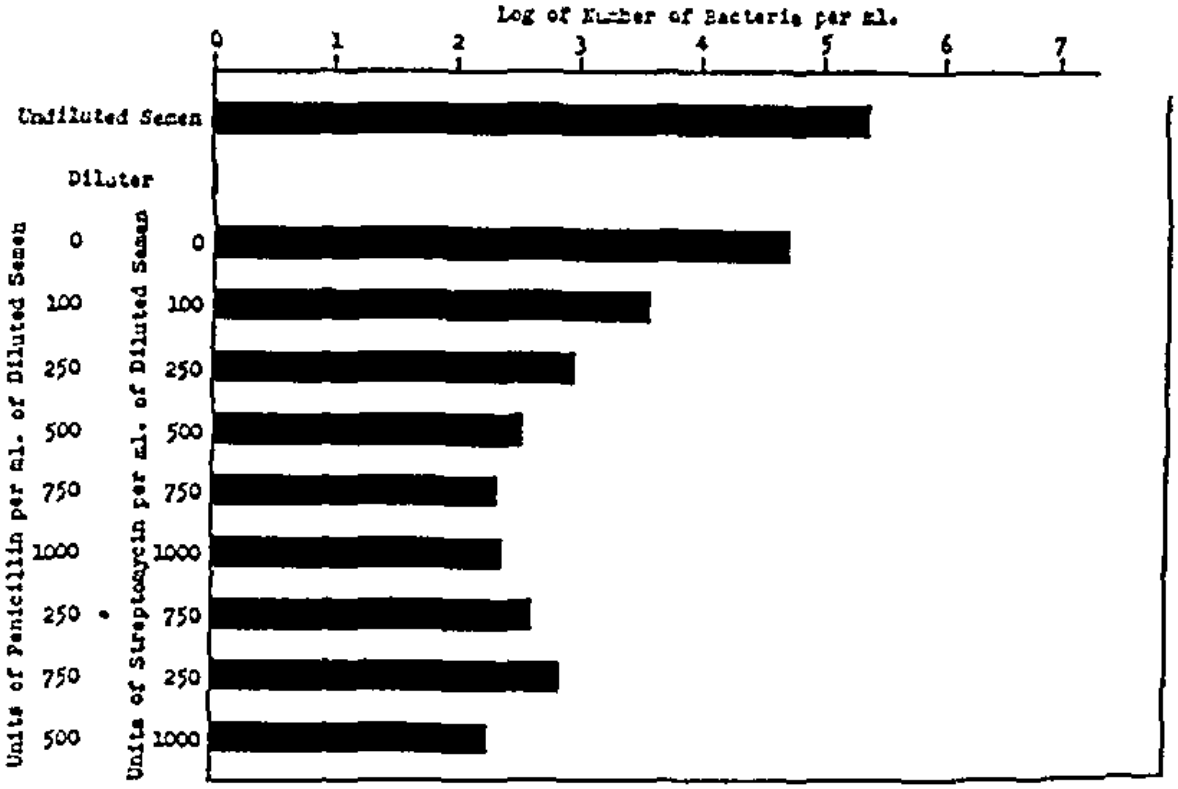

FIG 90. The effect of a combination of penicillin and streptomycin upon bacterial populations in freshly diluted semen inoculated with bacterial cultures (9).

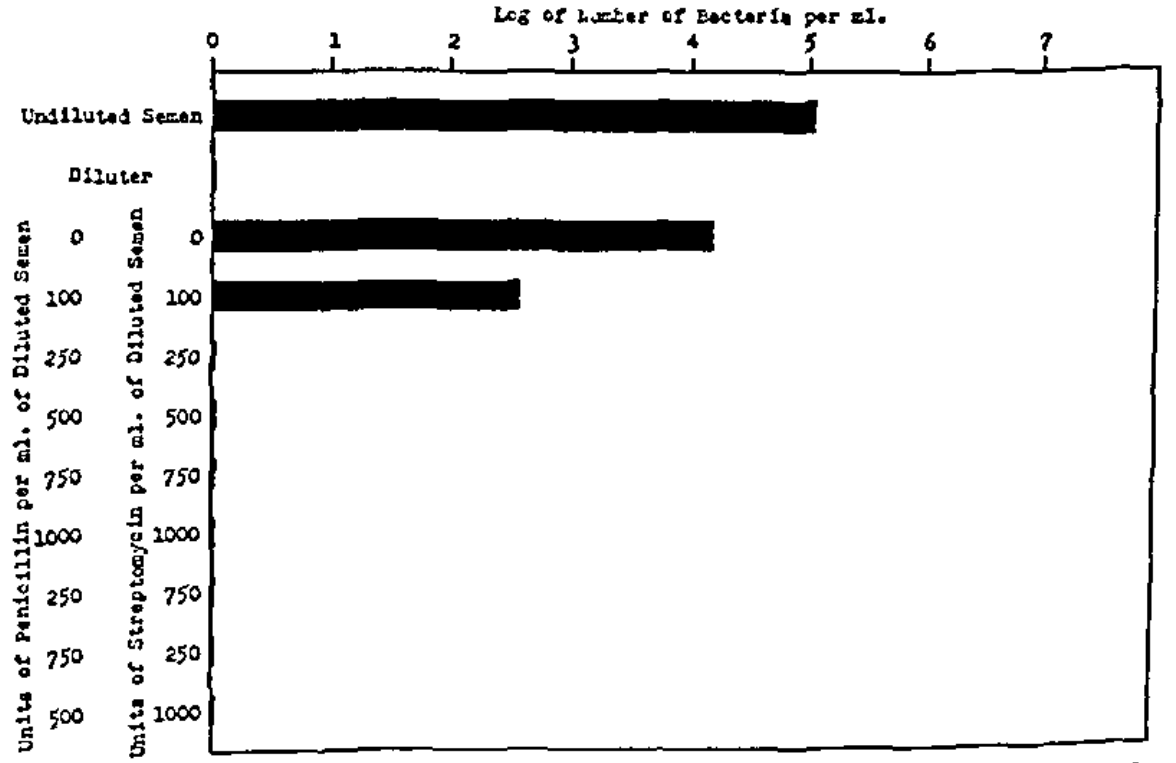

FIG 91. The effect of a combination of penicillin and streptomycin upon bacterial growth in inoculated, diluted semen stored for 8 days at 4.5°C (9).

., including gram-positive rods, gram-positive cocci, and
.e rods.

e 92 presents the average plate counts of the freshly diluted, inocu-
d semen plated within 1 hour following treatment with penicillin-streptomycin. The undiluted semen had a plate count of 270,000 bacteria per milliliter, while the diluted semen without the antibiotic averaged 52,000 per milliliter. There was a decrease to 4,000 organisms per milliliter when 100 units each of penicillin and streptomycin were added to the diluted semen. The number of bacteria per milliliter decreased as the concentrations of penicillin and streptomycin increased from 100 units of each to 1,000 units of each per milliliter. It is possible that better bacterial control of the fresh material was not obtained because the serial dilutions tended to dilute the penicillin-streptomycin concentrations below their most effective inhibitory level.

As shown in figure 91, there were marked decreases in the bacterial plate counts following storage for 8 days as compared with the same samples plated before storage (fig. 90). Negative plate counts consistently were obtained at levels above 100 units each of penicillin and streptomycin. Field studies recently completed indicate that negative plate counts also may be expected when diluted semen used for artificial breeding is treated with 1,000 units per milliliter each of penicillin and streptomycin (fig. 92). In these experiments the treated diluted semen was plated 24 to 32 hours after collection and dilution.

Desoxycholate agar plates showed that organisms of the coliform group also were effectively controlled by penicillin plus streptomycin (9).

Streptomycin in Artificial Insemination

From the practical standpoint, the usefulness of streptomycin can be determined only by the relative fertility of semen so treated. During 1948, Almquist and Prince (10) conducted a field trial in cooperation with Western Pennsylvania Artificial Breeding Cooperative, Clarion, Pa., to test the effect of streptomycin alone and in combination with penicillin upon the fertility of semen from bulls used for routine artificial insemination. Each diluted semen sample was divided into two equal portions. One portion remained untreated, while the other portion was treated with either 1,000 units of streptomycin or 1,000 units each of penicillin and streptomycin per milliliter of diluted semen. Thus each semen sample served as its own control. It was felt necessary to use this experimental design because of the wide variation in numbers and types of bacteria found in semen collected at various times from the same bull (2). Other published (11)

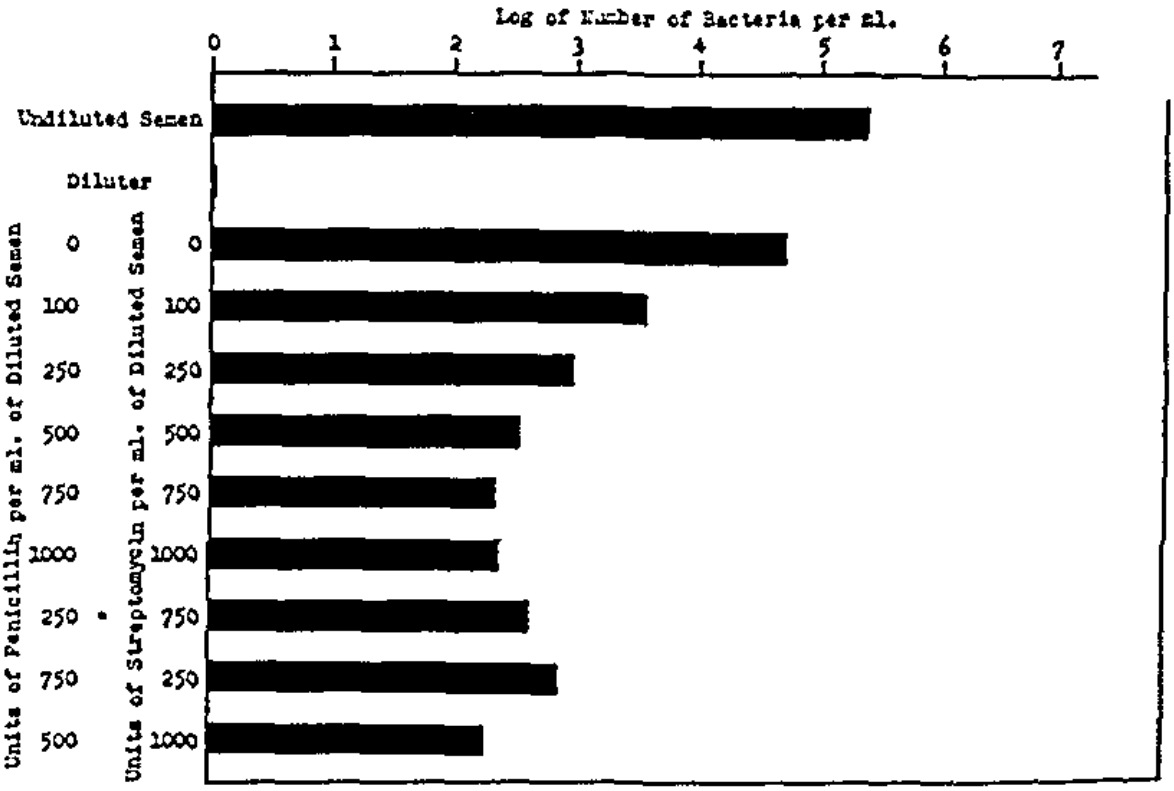

FIG. 90 The effect of a combination of penicillin and streptomycin upon bacterial populations in freshly diluted semen inoculated with bacterial cultures (9).

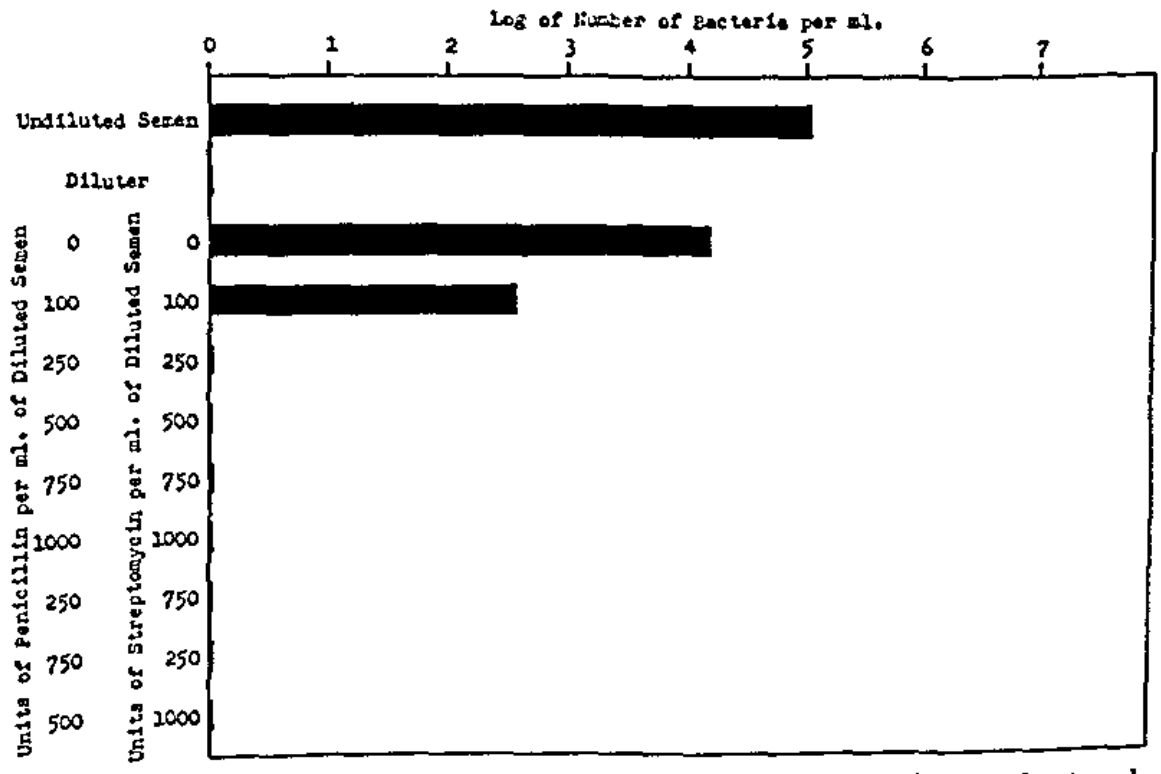

FIG. 91 The effect of a combination of penicillin and streptomycin upon bacterial growth in inoculated, diluted semen stored for 8 days at 4.5°C (9).

Results presented in table 87 indicate that streptomycin alone and combined with penicillin markedly improved the fertility of semen from five relatively infertile bulls. Streptomycin alone improved fertility by 67.3 per cent as compared with untreated controls. Penicillin and streptomycin together resulted in an increase of 55.1 per cent over the controls. Each of the five relatively infertile bulls showed a significant improvement in breeding efficiency when the semen was treated with antibiotics. Two other problem bulls failed to respond and were slaughtered by the Cooperative before completion of the experiment. Though the response in fertility is striking, it is necessary to caution that not all relatively infertile bulls will respond to such a marked degree. Many factors affect fertility, and it should not be expected that the use of antibiotics in semen will solve all breeding difficulties with bulls.

Limited data on two relatively fertile bulls (table 87) indicate that streptomycin either alone or in combination with penicillin does not aid fertility. In fact, the combination appeared to bring about a slight decrease in conception rate. Further fertility data on semen from additional relatively fertile bulls are needed before definite conclusions can be reached.

Summary

Streptomycin in concentrations of 250 to 1,000 units per milliliter of diluted bull semen inhibits bacterial growth without causing a significant decrease in the motility of the spermatozoa during storage. Levels of streptomycin above 1,000 units per milliliter are detrimental to the viability of bovine spermatozoa. The antibiotic is very stable in stored diluted semen.

Penicillin and streptomycin in combination are superior to either one alone for controlling the growth of bacteria in diluted semen. Field studies recently completed indicate that negative plates may be expected when diluted semen used for artificial breeding is treated with 1,000 units each of penicillin and streptomycin per milliliter. Concentrations of each antibiotic ranging from 100 to 1,000 units per milliliter of diluted semen do not affect the ability of the spermatozoa to remain motile during storage.

Streptomycin, or penicillin plus streptomycin, markedly improves the fertility of semen from certain relatively infertile bulls used for routine artificial insemination. Streptomycin, when used alone, increased the conception rate of the bulls of low fertility by 67.3 per cent, and, in combination with penicillin, by 55.1 per cent.

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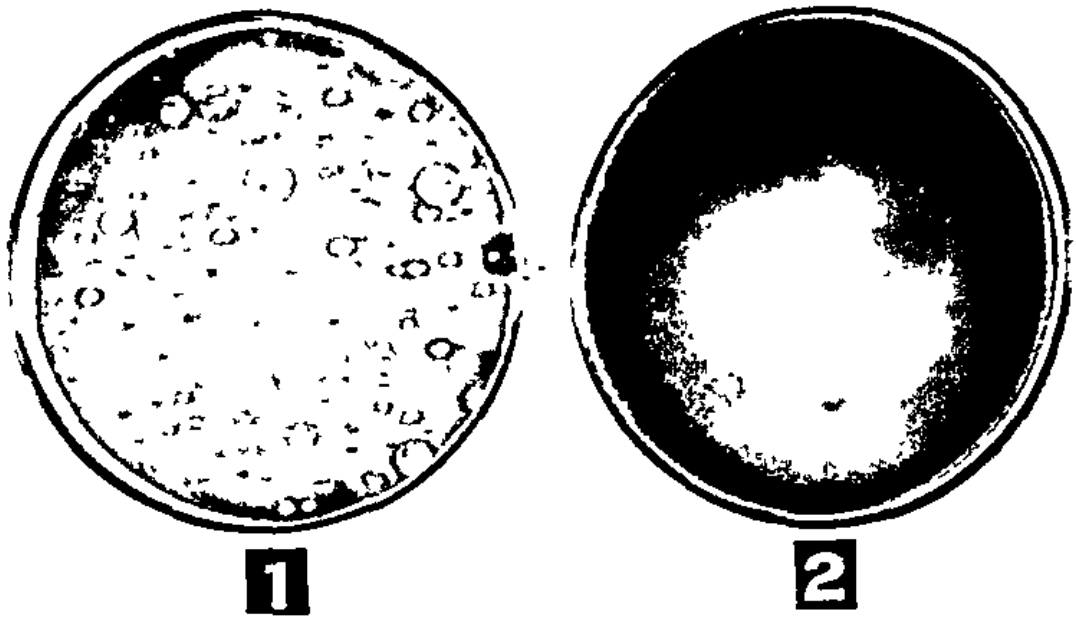

FIG 92. Control of bacteria in diluted bull semen with a combination of streptomycin and penicillin: 1 untreated diluted semen; 2. same diluted semen treated with 1,000 units each of streptomycin and penicillin (Original).

TABLE 87

Effect of streptomycin alone and in combination with penicillin upon the fertility of bull semen

TREATMENT OF DILUTED SEMEN	NUMBER OF INSEMINATIONS	CONCEPTION RATE*	DIFFERENCE
Five relatively infertile bulls			
Control	499	38.5	
Streptomycin	480	64 4	+25.9
Control	479	40 5	
Penicillin-streptomycin	476	62.8	+22 3
Two relatively fertile bulls			
Control	221	60.6	
Streptomycin	202	59.9	−0 7
Control	225	65 3	
Penicillin-streptomycin	229	58.1	−7.2

* Conception rate = per cent of cows which did not return for further service within 90 to 120 days following insemination

and unpublished (10) data suggest that 1,000 units of penicillin per milliliter of diluted semen is equally efficient in improving fertility of semen from certain bulls of lowered fertility.

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CHAPTER 46

USE OF STREPTOMYCIN IN AGRICULTURE

Many species of phytopathogenic bacteria have been found to be susceptible to the action of streptomycin. The practical application of streptomycin to agricultural problems, with the exception of veterinary medicine, has not been explored, however, to the same extent as it has been in the field of medicine. One reason for this was the high cost of streptomycin. Improved methods of streptomycin production, and the fact that a high degree of purification may not be necessary for many agricultural uses of this antibiotic, may lead to the introduction of streptomycin in phytopathology and related fields. Since streptomycin does not exhibit any fungicidal properties (1), its use in phytopathology may be limited to control of bacterial diseases of plants such as those caused by seed and surface-borne organisms.

Brown and Heep (2) were able to eliminate *X. pruni*, the cause of a serious disease of stone-fruit plants (*Prunus*), from infected budwood by soaking the bud sticks in a streptomycin solution. Ark (3) reported successful disinfection of cucumber seed contaminated with *Ps. lachrymans* and demonstrated the efficiency of streptomycin for fourteen species of phytopathogenic bacteria, both gram-positive and gram-negative. *S. scabies*, the causal agent of the potato scab disease, was found to be very susceptible to *crystalline* streptomycin. *Ag. tumefaciens*, the crown gall organism, showed a considerable resistance to streptomycin, in comparison with other bacterial species in the test. The practical application of streptomycin to control crown gall was suggested by Brown (4) and Hampton (5). The latter reported cures of both soft and hard crown gall, on numerous species of plants by applying a cotton-wool pad saturated with the antibiotic; by immersion of the galls; and also by injection with hypodermic syringe. The galls were eliminated in such important agricultural plants as *Prunus domestica*, *P. salicina*, *P. cerasus*, and *Pyrus communis*. This investigation suggested the possible value of streptomycin in nurseries where crown gall at times takes a large toll of plants. The ring-rot organism of potatoes, *C. sepedonica*, a gram-positive bacterium, was successfully killed *in vitro* and *in vivo* by Van Schaack (6), who tested Pontiac and White

3. Gunsalus, I. C., Salisbury, G. W. and Willett, E. L. Jour. Dairy Sci., 24: 911–919. 1941.
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They were then planted in garden soil in pots. No deviation from normal plants was noticed during 2 months in the greenhouse. This demonstrates a remarkable nontoxicity of the streptomycin for higher plants. The presence of the streptomycin was demonstrated in the embryo of the treated cucumber seeds. Embryos of the treated cucumber seeds, which were stored at room temperature for 2 months, still showed the presence of the antibiotic when tested on plates seeded with *Ps. lachrymans*. *E. carotovora*, the organism causing soft rot of carrots, potatoes, and a host of other fleshy plants, was successfully checked with the streptomycin when White Rose and Netted Gem potato slices were inoculated with a broth culture of the

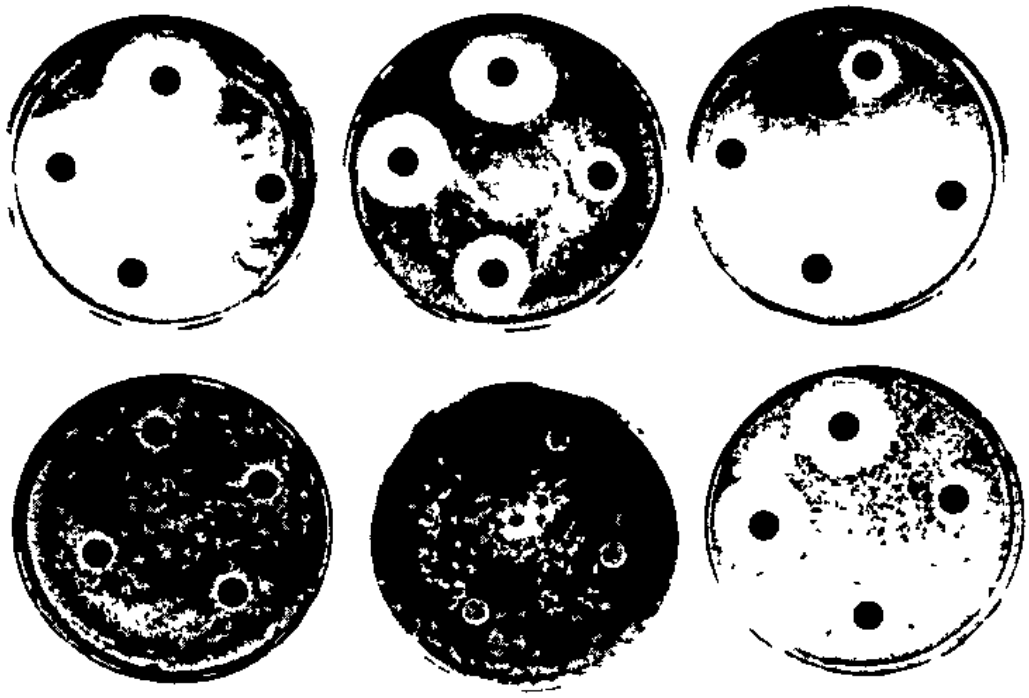


FIG 94
phytopatho
Ag. tumefac
F. cucurbit
clockwise. 1:10,000; 1:50,000; 1:100,000, and 1:1,000,000 (Original).

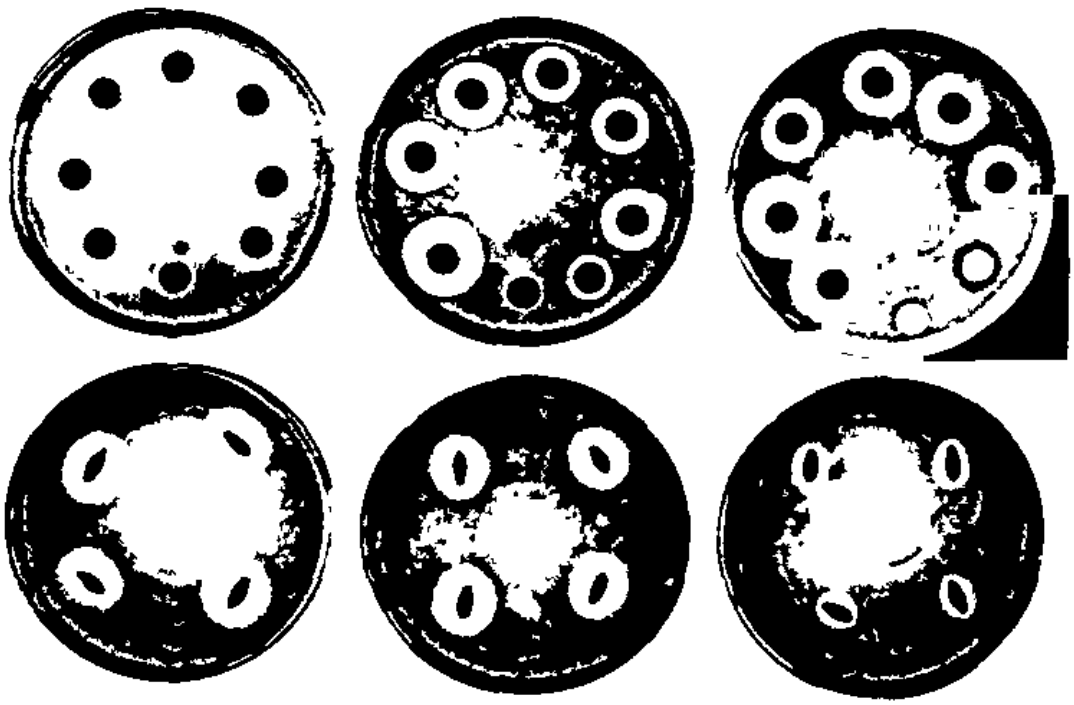
organism by placing on them sterile filter paper discs dipped in a 24-hour-old culture of *E. carotovora* and incubating them 1 to 6 hours before treatment with streptomycin (fig. 95).

Under greenhouse conditions, streptomycin appears to remain stable long enough to give some control of the leaf-spot organism, *Ps. punctulans*, on tomato plants, *Lycopersicum esculentum*. Lots of eight small tomato plants, variety Pearson, were used for the experiment. In one series eight plants were sprayed with the antibiotic 1 hour before inoculation with the

Rose varieties. No data on attempts to culture the treated seed pieces for ring-rot bacteria were presented in the report.

The author (7) made extensive studies on the effects of streptomycin on phytopathogenic bacteria under laboratory, greenhouse, and field condi-

to the genera *Erwinia*, *Xanthomonas*, *Corynebacterium*, *Pseudomonas*, and *Agrobacterium*. Figures 93 and 94 show inhibition zones, on plates seeded



F
geni
gane
Ph..
bein
plate from lower center disc, clockwise· 1 10,000; 1·100,000, and 1 1,000,000 (Original)

with phytopathogenic bacteria, due to different concentrations of the crystalline streptomycin. Cucumber seeds soaked in concentrations of the streptomycin varying from 1 to 10,000 to 1 to 1,000,000, dried at room temperature and placed on plates seeded with the angular leaf spot organism, *Ps lachrymans*, showed antibiotic activity as shown in figure 95, bottom row. It is interesting to note that the viability of the seeds was not affected by soaking the seed in the streptomycin for 24 or more hours. In one experiment the seeds were left in a solution until they germinated and the seedlings had grown to a large size forming an abundance of roots.

and untreated tomato seed was drilled by a grower. Counts were made on 200 plants in each series. There was no canker in the lot from treated seed, whereas the check showed 60 per cent canker. It appears that the streptomycin controlled the seed-borne diseases in both the cucumbers and the tomatoes. Since the tomato test was performed only once, the experiment should be repeated under a variety of conditions before conclusions are reached as to the value of the antibiotic as a control measure for tomato canker.

A few experiments on the use of streptomycin to control plant diseases indicate the possible application of this antibiotic in phytopathology, the limitations being its ineffectiveness against fungus diseases and its inactivation under field conditions when placed on plants. Streptomycin may be useful in controlling seed-borne bacterial diseases of plants.

TABLE 88

*Effect of streptomycin spray on bacterial speck of tomato**

TIME OF INOCULATION	NUMBER OF DISEASED PLANTS	NUMBER OF HEALTHY PLANTS
1 hour before treatment	1	7
24 hours before treatment	2	6
Check. Plants inoculated at start. No treatment	7	1
Check. Plants inoculated 24 hours after start of experiment	8	0
Check. Plants sprayed with distilled water	0	8

* Eight plants were used in each series.

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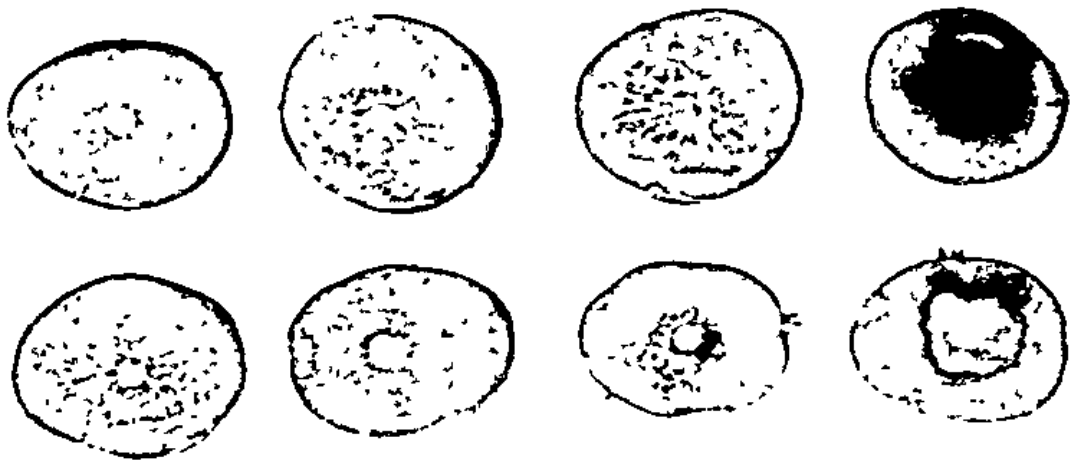
4. Brown, J. G Phytopath., 38: 3 1948
5 Hampton, J. E. Phytopath , 38 11–12. 1948.
6 Van Schaack, V. Phytopath , 38 27. 1948.
7 Ark, P A. Unpublished

ADDENDUM

A number of other infectious diseases, not listed in this volume, have been reported to respond favorably to treatment with streptomycin. Unfortunately, either the number of cases was too limited or the results were too inconclusive to justify broad generalization.

pathogen; another series was sprayed with the antibiotic 24 hours before the organism was applied. The experimental plants were kept in a greenhouse at average temperature of 75°F, and the humidity of the air was maintained at a high level. Under these conditions the check plants developed the first symptoms of the speck in 4 days. No symptoms of the disease were observed on treated plants during 30 days of observation. In the experiments designed to learn whether the disease could be eradicated by streptomycin, inoculation was made 1 and 24 hours respectively before spraying with the antibiotic. From the results (table 88) it appears that streptomycin can prevent the disease under greenhouse conditions. Under field conditions, however, streptomycin 1 to 1,000,000 completely failed to control walnut blight caused by *X. juglandis*.



Streptomycin (Original).

To evaluate streptomycin as a seed disinfectant, cucumber seeds, field-contaminated with *Ps. lachrymans*, were used in greenhouse experiments, and tomato seeds, field-contaminated and artificially contaminated with the canker organism, *C. michiganense*, were used in a field trial. The treatment consisted in soaking the seeds in the streptomycin solution, 1 to 10,000, for 20 minutes. Seeds were dried before planting. At the end of the experiment, the cucumber plants from seeds treated with the streptomycin were free of the disease, whereas the untreated lots showed 5 per cent infection. A total of 1,000 cucumber seeds were used for each series in 10 separate trials. Four ounces each of treated and untreated tomato seed was used for each series in 10 separate trials. Four ounces each of treated

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ADDENDUM

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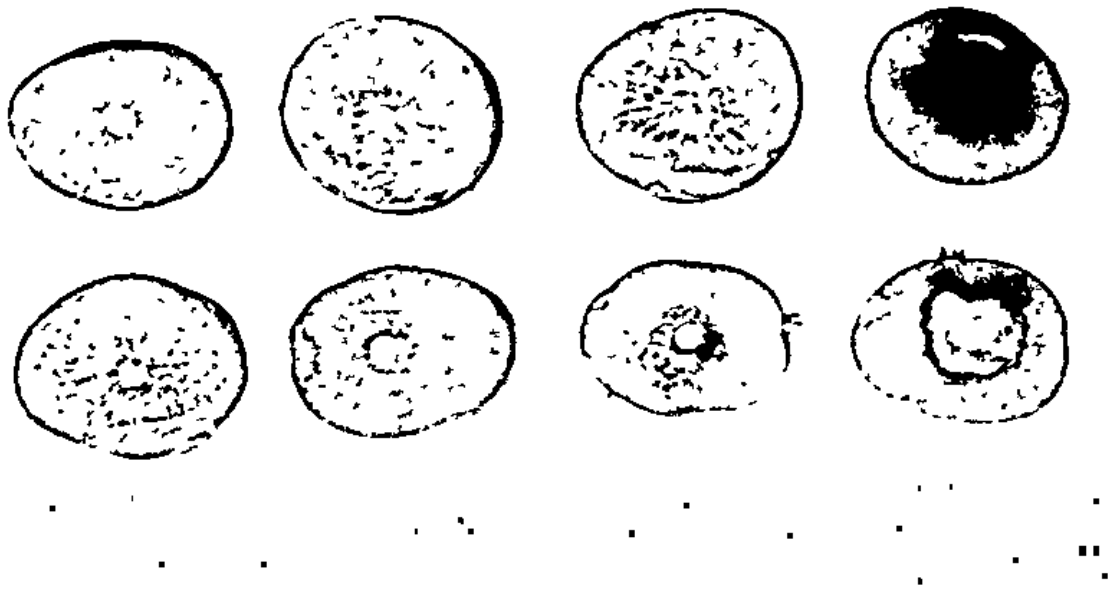

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Two such cases may be mentioned here. "Pinta," "mal del pinto," or "carate" is a disease that first produces on the face, arms, and legs spots which later extend into larger pigmented lesions all over the body. The causative agent of this disease was designated by Brumpt (1) as *Treponema carateum*. Olarte and Varela (2) treated one case in Mexico by giving sixteen injections of streptomycin, 0.5 gm every 12 hours. The treponemas were rapidly paralyzed, some within 15 minutes after the first injection, as shown by dark-field illumination; they disappeared completely after 48 hours. This reaction was accompanied by disappearance of the erythema and squamous lesions and gradual fading of the pigmented lesions. Similar clinical results were obtained with a group of other patients.

Experimental work with hamsters and dogs indicates that, although streptomycin and penicillin both are effective against leptospiremia and may be lifesaving for dogs and hamsters, only streptomycin successfully combats leptospiruria and hence the carrier state. Because accurate diagnosis is usually delayed until the leptospirae have disappeared from the blood, streptomycin increases in importance as a therapeutic and preventive agent in canine leptospirosis (3).

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